MSc.-Thesis

TOWARDS THE SYNTHESIS OF D-TAGATURONATE

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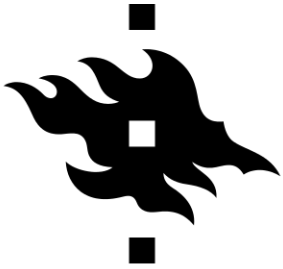

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Concerns about the state of the environment and the global climate change has created a need for more efficient and greener ways to produce chemicals and fuels. One solution to these challenges is to find improved ways of utilizing biomass.

This thesis deals with the valorization of pectin rich biomasses. Bioengineering yeast to express the alternative galacturonic acid catabolism pathway opens up an opportunity to use these underutilized biomasses in a more efficient way. In order to bioengineer yeast, understanding of the metabolic pathways and the enzymes functioning on these pathways is required. In order to map out certain degradation steps, access to non-commercial compounds and structural analogues is a necessity. The aim of this thesis was to develop a synthetic route to D-tagaturonate, and its structural analogues, which are intermediates on the galacturonic acid catabolism pathway.

The chosen multistep synthetic route to D-tagaturonate proved to be challenging. The end product was not obtained, however, the laboratory work showed that the synthetic route is feasible after some minor adjustments. On a general level, new information on the limitations of widely utilized protective groups could be uncovered. These findings will help to optimize the synthetic route to D-tagaturonate. In addition, these findings show that there is still room for improvement in orthogonal protective group strategies applicable to the synthesis of complex organic molecules such as carbohydrates.

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List of Abbreviations

Ac	acetyl
ACN	acetonitrile
ATP	adenosine triphosphate
$BF_3 \cdot OEt_2$	boron trifluoride etherate
Bn	benzyl
CSA	Camphorsulfonic acid
DCM	dichloromethane
DMP	dimethoxypropane
FGI	functional group interconversion
HF	hydrogen fluoride
PG	protecting group
PPTS	Pyridinium *p*-toluenesulfonate
r.t	room temperature
TES	Triethylsilyl
NBS	N-bromosuccinimide
NMR	Nuclear magnetic resonance
TBDMS	*Tert*-butyldimethtylsilyl

1 INTRODUCTION

Concerns about the state of the environment and the global climate change has created a need for more efficient and greener ways to produce chemicals and fuels. One solution to these challenges is to find improved ways of utilizing biomass as a starting material for the production of chemicals and fuels.

Microorganisms play a key role in the conversion of biomass to fuels and chemicals. Yeast is used in the fermentation of sugars to bioethanol and algae/bacteria are utilized in the production of hydrogen and biogas respectively. There is a growing need for more efficient tools for the processing of biomass. While several companies are currently investing major funds in the production of added value products from industrial side streams, a lot more needs to be done. One alternative is to bioengineer microorganism which would allow a more efficient and environmentally friendly alternative for the conversions of low value biomass into useful products or intermediates. The design of suitable bioengineered microorganisms requires a detailed understanding of their metabolic pathways. In order to map out certain steps on these pathways, access to non-commercial compounds and structural analogues is required.

This thesis deals with a biomass valorisation with special focus on pectin rich biomass. Pectin is an abundant polysaccharide that is currently produced by the food industry as a side waste product. Bioengineering yeast to express an alternative galacturonic acid degradation pathway would allow degradation of pectin and provide a means to produce higher value products from this underutilized biomass. In order to bioengineer yeast, information on the enzymes functioning on the metabolic pathways is needed. In this work I set out to synthesize one of the key intermediates on the degradation pathway, D-tagaturonate, and its structural analogues. In the literature review of the thesis, the basic principles of the bioengineering aspects is provided together with a more detailed overview of the principles of carbohydrate chemistry. In the results and discussion section, the challenges faced on the synthetic routes are addressed.

2 FUNGI: CENTRAL METABOLISM AND APPLICATIONS

Fungal species are the primary decomposers of organic material which is why they are able to degrade and process a wide range of organic molecules. [1] Fungi produce extracellular enzymes which degrade biomolecules to smaller organic compounds. In addition to the degradation, fungi are able to take up and metabolize the formed compounds. [2] This process leads to primary and secondary metabolites. [3] The primary metabolites are essential for keeping the fungi alive, while secondary metabolites include compounds with other functions as well. [4] The secondary metabolites can be exploited for commercial use as shown by the examples in table 1. [5] [6].

Table 1. Examples of commercial fungal metabolites. [5] [6]

Metabolite	Fungal species	Application
Cephalosporins	*Acremonium Chrysogenum*	Antibacterial
Gibberellins	*Gibberella fujikuroi*	Plant hormone
Griseofulvin	*Penicillium griseofulvum*	Antifungal
Zearalenone	*Gibberella zeae*	Cattle growth

In addition to the examples provided above, the appealing properties of fungi has led to their use in the industrial production of enzymes, vitamins and pigments. [7]. *Saccharomyces cerevisiae* is used in the fermentation of carbohydrates to bioethanol [8] and *Pichia pastoris* is used in the production of recombinant proteins. [9] In figure 1 the central metabolism of fungi is presented together with the biosynthesis of macromolecules and secondary metabolites [5] [10].

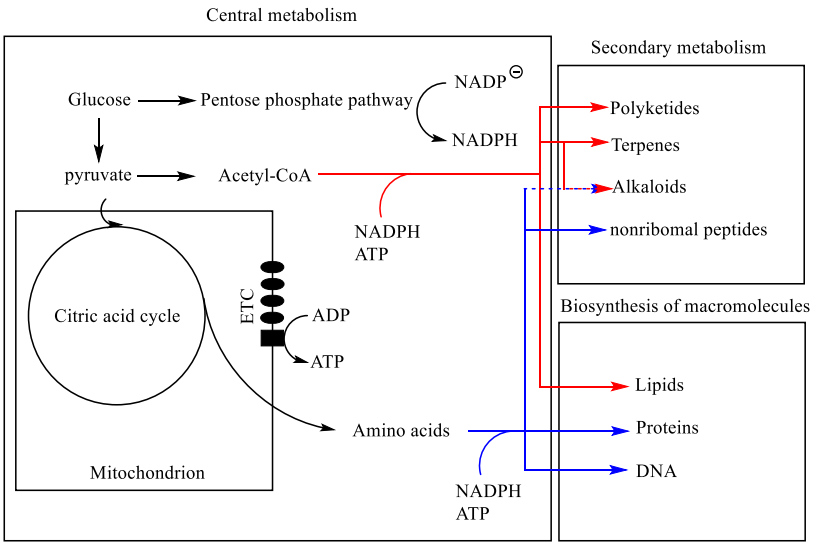


Figure 1 The central metabolism of fungi is displayed together with the biosynthesis of macromolecules and secondary metabolites. Adapted from Nielsen et al. [5] [10].

2.1 Bioengineering of the fungal metabolism

Bioengineering of fungal strains is required in order to enhance the conversion of organic materials [11] or to alter the substrate scope of fungi [12]. This is especially true when considering the production of commercially relevant secondary metabolites as these are rarely obtained in sufficient quantities under suboptimal conditions without metabolically engineered fungi [5]. Bioengineering can also be used to express genes that fungi don't naturally have or to delete expression of natural genes [13]. Techniques such as a CRISPR/Cas9 [14] and computational strategies [15] are used in bioengineering of fungi.

2.2 Pectin degradation in fungi

Pectin is an abundant unutilized biomass that is produced in large amounts in the food industry and considered as waste. For example, orange peel waste has a pectin content of 247 mg/g of dry matter [16]. Because Pectin-rich biomasses is not efficiently utilized it is a feedstock of interest for the production of biofuels and chemicals [17] [18]. Pectin has many different roles in plant biology. It is part of the cell wall structure and plays a role during plant growth [19]. The pectin is a polysaccharide containing a backbone of α-(1 → 4)-linked galacturonic acid units. The pectin polysaccharides are divided into homopolysaccharides and heteropolysaccharides of which homopolysaccharides are more abundant in nature [19]. The carboxylic acid functional group in the galacturonic acid residues are largely methylated in the homopolysaccharide series. In addition, acetyl groups are known to occur on positions 2 and 3 [20]. The pectin heteropolysaccharides contains L-Rhamnose in their backbone and D-galactose, L-arabinose, D-glucuronic acid and D-xylose in their sidechains [21]. In figure 2 selected structural features of pectin are presented [21] [20] [19].

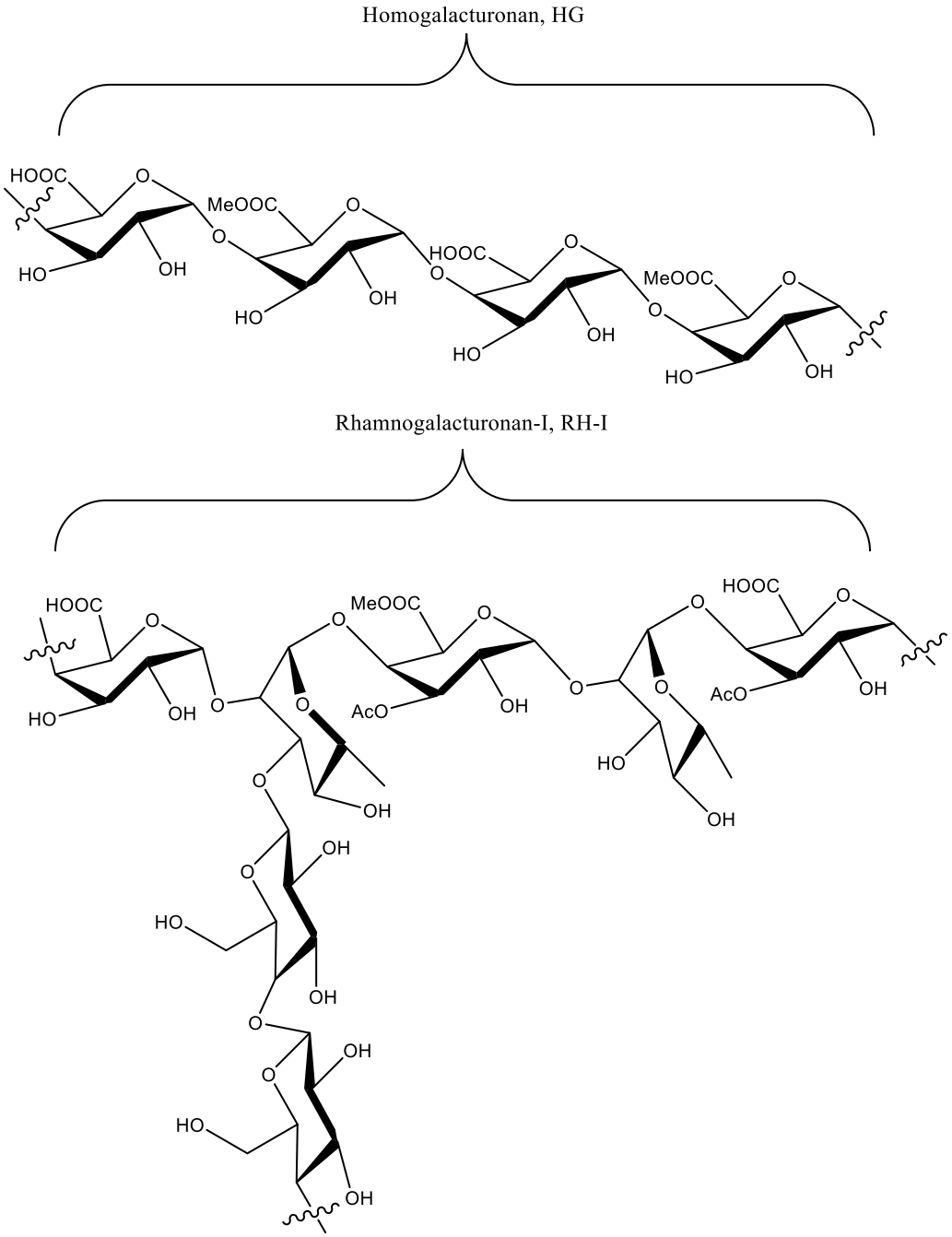


Figure 2 The molecular structures of homogalacturonan and rhamnogalacturonan are displayed [21] [20] [19].

Galacturonic acid is an important carbon source for fungi and bacteria which live on decaying plant material [19]. Pectin rich materials can be converted for example to ethanol by using fermentation by *S. cerevisiae*. The problem with using pectin rich materials in the production of added-value products is that the robust species such as *Cerevisiae* are unable to use naturally occurring D-galacturonic acid as a substrate [17]. Bioengineering *S. cerevisiae* to use fun-

gal [22] or bacterial [23] metabolic pathways would solve this challenge. The fungal and bacterial metabolic pathways for galacturonic acid degradation differs from each other and these pathways are presented in figure 3. [23] [24]. It has already been proven that these metabolic pathways can be engineered in order to produce intermediates on them [13]. In order to predict the outcome of bioengineering, insights into the substrate scope and tolerability of the enzymes operating at each step is required. In this work, the focus is not on the bioengineering aspects but on the synthesis of one of the key-intermediates on the bacterial metabolic pathway in order to map the substrate scope tolerability of the enzyme operating at this step. Therefore, a more detailed chapter focusing on the chemical aspects will be provided next.

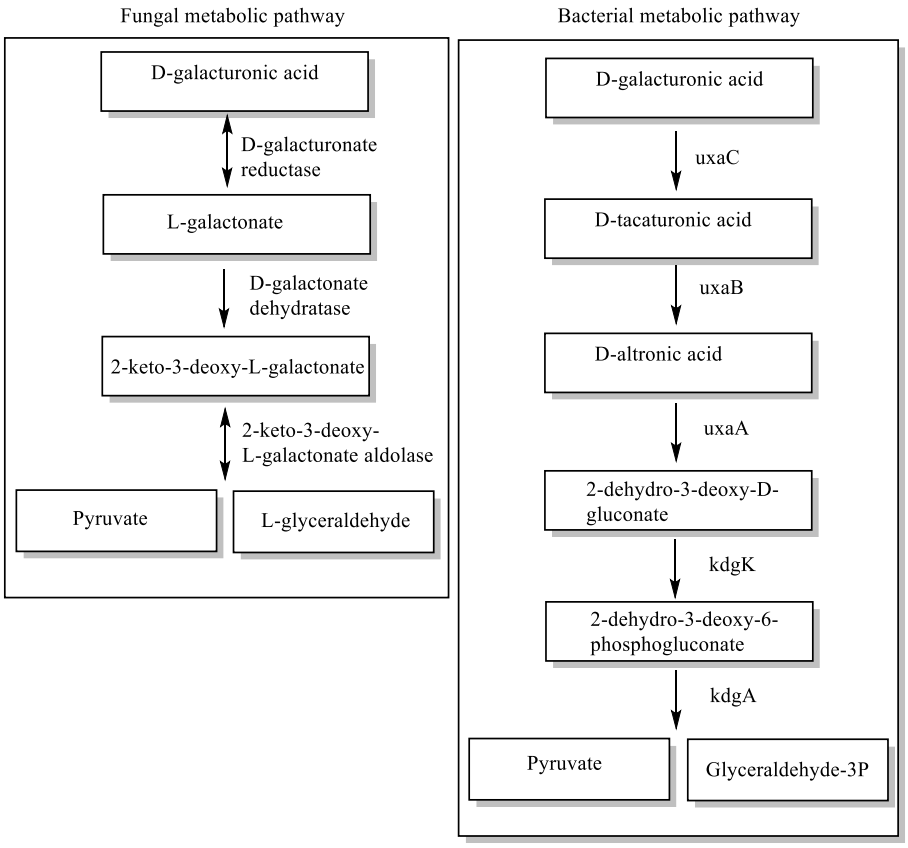


Figure 3 Fungal and bacterial metabolic pathways for catabolism of D-galacturonic acid [23] [24].

3 CARBOHYDRATE CHEMISTRY

Carbohydrates are one of the four major molecules of life, the other three being lipids, proteins and nucleic acids. Carbohydrates are classified as mono-, oligo-, and polysaccharides depending on the number of sugar monomers in the carbohydrate chain. Carbohydrates can also be attached to other biomolecules and such structures are termed glycoconjugates. [10]. In figure 4 examples of different carbohydrates are presented. Glucose is a monosaccharide that is used as an energy source. Glucose and other monosaccharides are also utilized as a carbon source in biosynthesis of other molecules, e.g. lipids and amino acids. Cellulose is an example of a polysaccharide. It is an essential part of the plant cell walls and the most abundant biomolecule on earth [10]. The ABO-blood group antigens are oligosaccharides present on the cell surface of red blood cells [25]. The Tn-antigen is a glycopeptide which can be utilized in the diagnostics of cancers [26]. These examples highlight the important roles that carbohydrates play in nature.

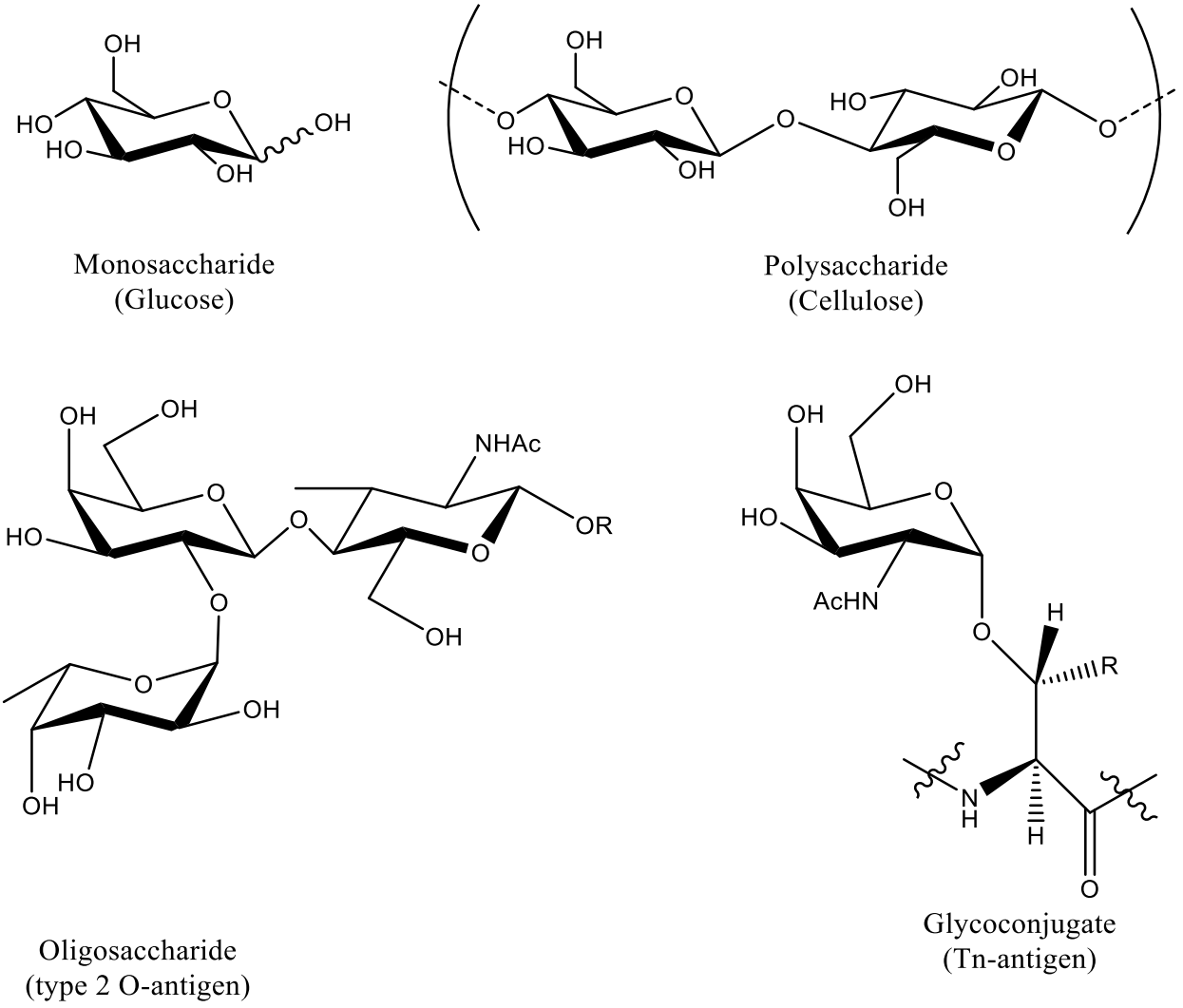


Figure 4 Examples of carbohydrates

3.1 Intrinsic properties of carbohydrate – structural aspects

Carbohydrates are polyhydroxylated ketones (ketoses) or aldehydes (aldoses). They contain a minimum of three carbon atoms in their backbone. The most simple carbohydrate is glyceraldehyde which can exist either as the D- or L-enantiomer depending on the configuration at the stereocentre (figure 5) [27] [28].

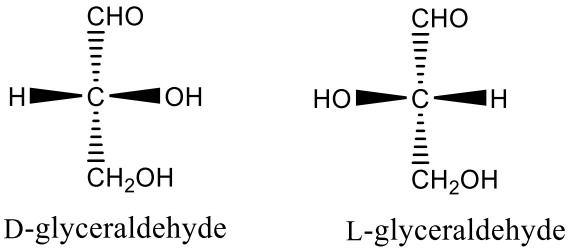


Figure 5 Fischer projection of D- and L-glyceraldehyde

Increasing the chain length of the backbone provides the corresponding L- and D-series of aldoses [28]. As the chain grows, the number of stereocenters and stereoisomers increase. A molecule with n stereocenters gives rise to 2^n stereoisomers. There are e.g. sixteen aldohexoses [29]. The ketoses follow a similar pattern as the aldoses and the simplest ketose is 1,3-dihydroxy acetone. Due to the ketone functionality, the ketoses have fewer stereoisomers than aldoses. Selected examples of aldoses and ketoses are presented in a figure 6 [28].

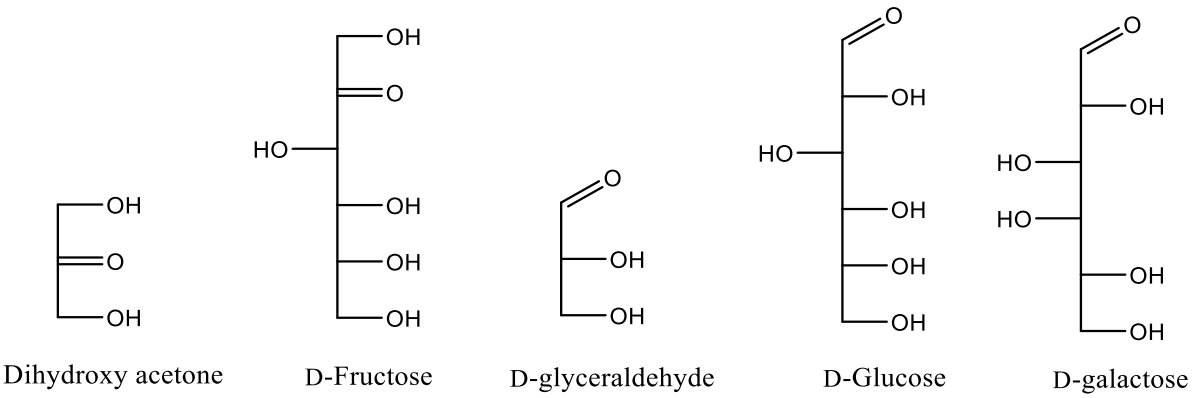


Figure 6 Selected aldoses and ketoses [28].

3.1.1 **Ring formation and mutarotation**

Carbohydrates can form rings due to the possibility of an intramolecular reaction between a hydroxyl group and a carbonyl group. Five- and six membered rings are especially stable in their nature and the heterocycles formed by carbohydrates are termed furanoses (five re-membered ring) and pyranoses (six membered ring) [30] [29]. In a solution, carbohydrates exist in an equilibrium between cyclic and acyclic forms. For example, D-glucose exist mainly in pyranose forms (> 90%) in solution (see figure 7). In fact, the acyclic forms are present only in small quantities (< 1%). When pyranoses/furanoses form, the nucleophilic attack of the hydroxyl group/alkoxide ion can occur from the α- or β-phase thereby giving rise to two distinct hemiacetals termed anomers [28]. Transition from one anomer to another proceeds through the acyclic intermediate. This solution behaviour of carbohydrates is called mutarotation. The rate of mutarotation is accelerated by acids and bases and the proportions of the different species in the equilibrium is affected by the applied conditions [30] [28].

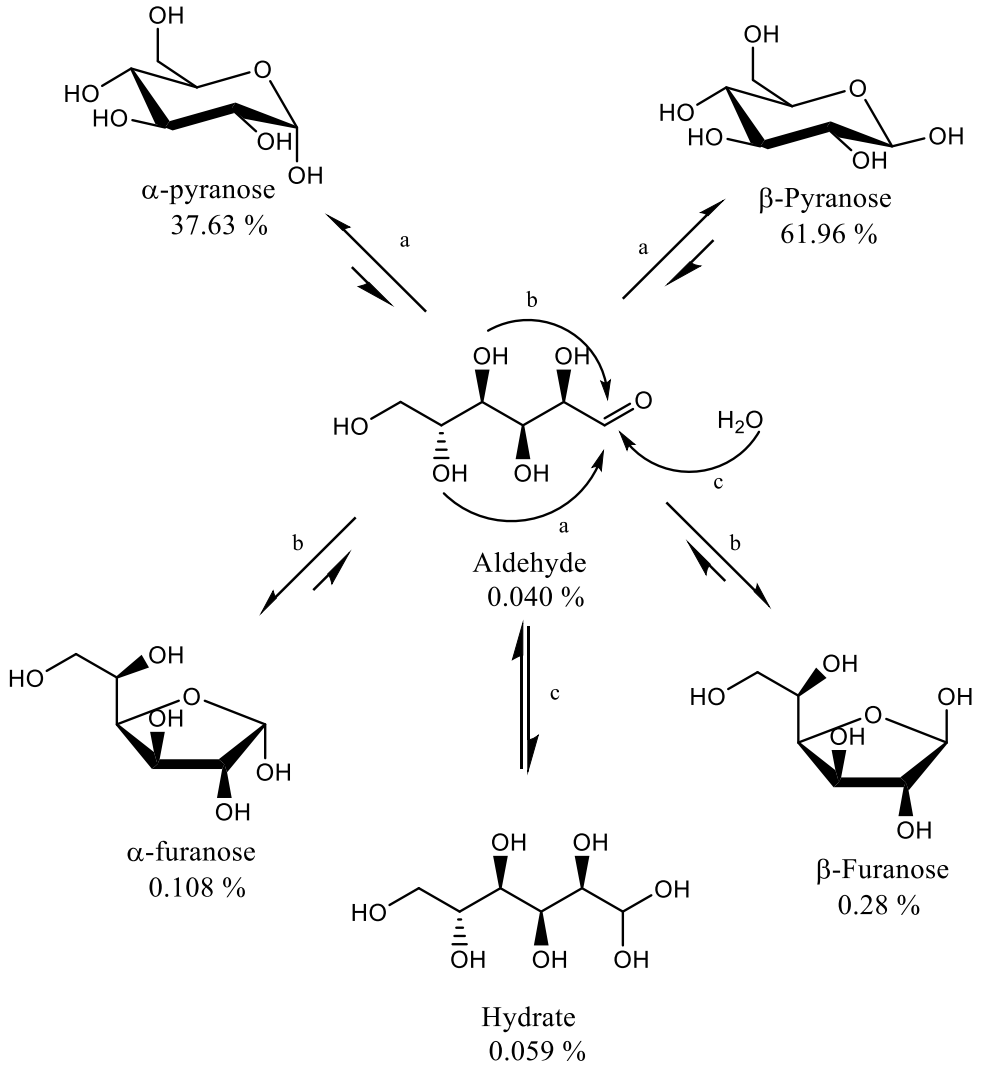


Figure 7 Mutarotation of D-glucose and percentages of conformations in aqueous solution [30].

3.1.2 **The anomeric effect**

The anomeric effect was first observed by J.T. Edward and R.U. Lemieux in the 1950s [31]. The term "anomeric effect" was originally used to describe situations in which anomeric substituents on pyranoses prefer positions which are not explainable by pure steric considerations [32]. For example, a substituent on cyclohexane would prefer an equatorial position while a substituent at the anomeric center would prefer an axial position (see illustration in figure 8) [33].

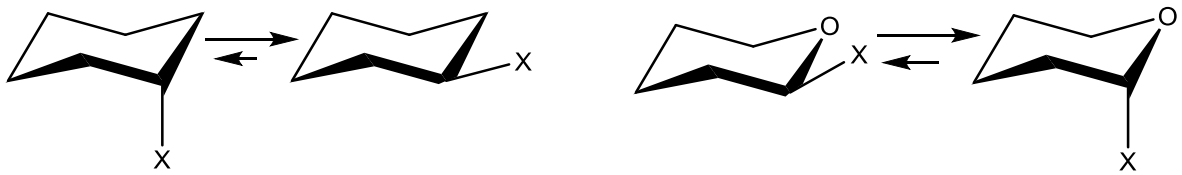


Figure 8 Preferred conformations of substituent at cyclohexane and pyranose.

There are several different hypotheses which seek to explain the anomeric effect. Regardless of the hypothesis employed, both steric and electronic factors affect the preferred conformation and external factors such as solvents also play a role. While there is still a lively debate on the underlying reasons of the anomeric effect, two of the most used explanations will be provided here (dipole-dipole; hyperconjugative). Other explanations also exist e.g. non-classical hydrogen bonding [33] [34] [35].

The dipole-dipole model explains the preference of one anomer over the other by the difference in their dipole moments [32]. A substituent in an axial position would have a smaller dipole moment compared to a substituent in an equatorial position. This would lead to an increase in stabilization in a non-polar solvent (figure 9) [34] [35].

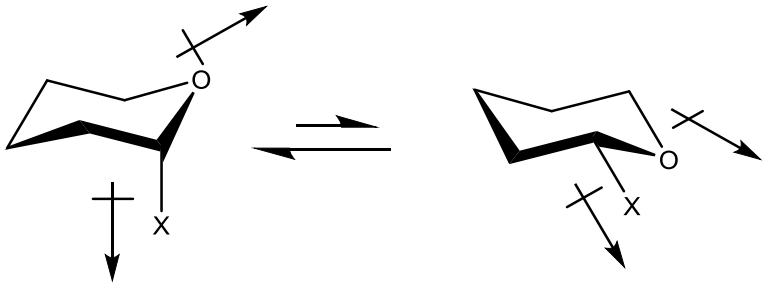


Figure 9 Pyranose rings equilibrium between conformations with different dipole moments. Dipole vectors of the conformations are presented with arrows.

The hyperconjugative model, the anomeric effect is explained as a partial delocalization of the ring oxygens free electron pair into the anti-bonding orbital at the anomeric center. This orbital overlap would only exist when the substituent is in an axial position (figure 10) [32] [34].

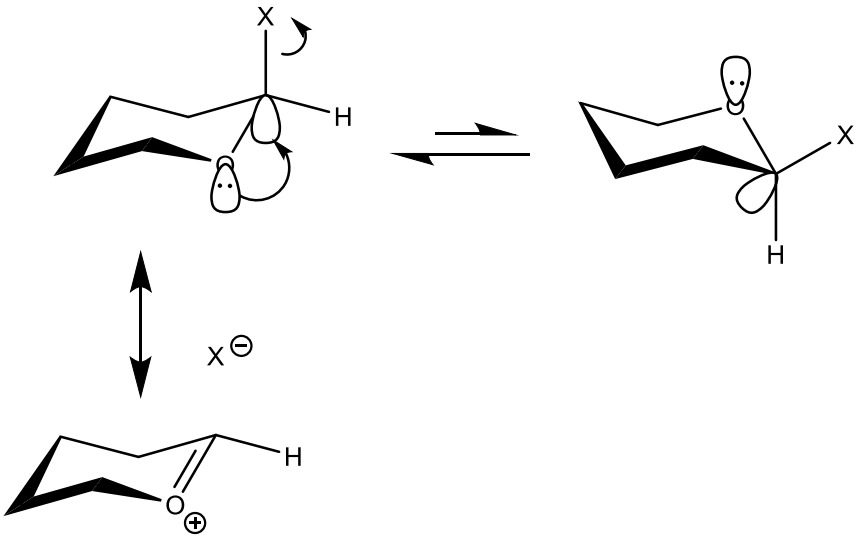


Figure 10 Hyperconjugative interaction.

The overall anomeric effect is more complex than the above discussion implies as it can be further divided into *exo* and *endo* contribution. The *endo* anomeric effect was discussed above, however, the *exo* anomeric effect deals with the preferred conformation of substituent (gauche vs antiperiplanar). The *exo* anomeric effect deals with delocalization of the *exo*-cyclic oxygens free electron pair. This effect is stronger in cases where the *endo* and *exo* anomeric effects do not compete with each other [32].

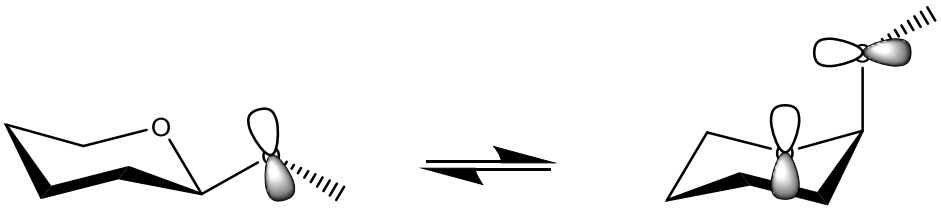


Figure 11 Delocalisation of exocyclic oxygens free pair [32].

3.1.3 **Conformations of monosaccharides**

The pyranose and furanose rings of carbohydrates can adopt numerous conformations in solution. To distinguish between the different conformations six main conformational classes are used: C (Chair), B (Boat), S (Skew), H (Half-chair), E (envelope) and T (Twist). Some examples are given in figure 12. While a large number of conformations are possible for the furanoses and pyranoses (a total of 20 for furanoses and 38 for pyranoses), pyranoses generally prefer the $^{4}C_{1}$ or $^{1}C_{4}$ conformations and furanoses ^{2}E, E_{2}, ^{3}E or E_{3} conformations [36] [37] [38] [39] [40].

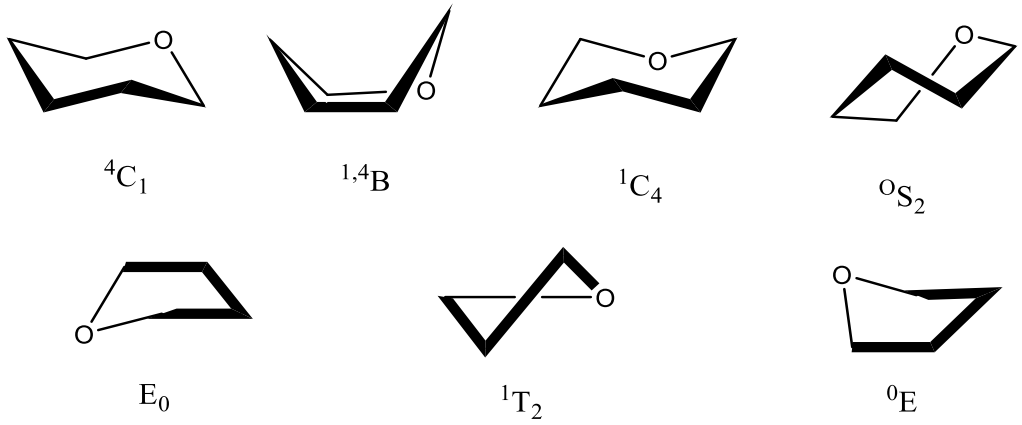


Figure 12 selected conformations of pyranose and furanose rings [38].

The conformation adopted by carbohydrates in solution is governed by steric, electronic and solvent effects [41]. For example, the free energy difference between $^1C_4$ and $^4C_1$ conformations of β-D-glucopyranose is 24 kJ/mol. As can be seen from figure 13, this is due to unfavourable steric clashes between substituents in the $^1C_4$-conformation. [42].

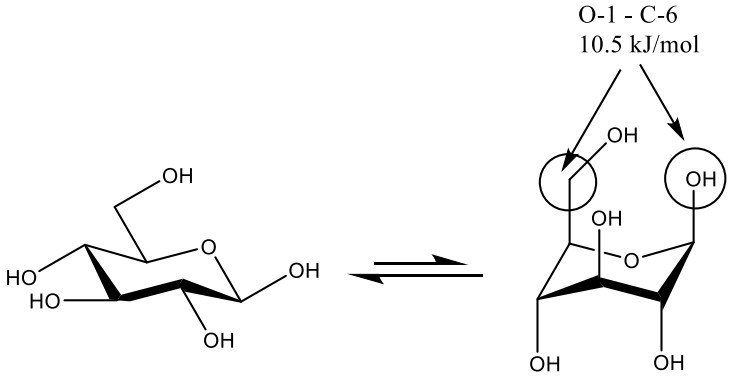


Figure 13 Equilibrium between $^1C_4$ and $^4C_1$ conformations of β-D-glucopyranose. Interaction between O-1 and C-6 are marked in the $^1C_4$ conformation as an example of destabilizing axial interaction [42].

The conformation of a sugar affects its reactivity and even conformations that are normally unstable may serve as reaction intermediates [43]. In addition, the positions of hydroxyl groups (axial vs equatorial) are closely related to their reactivity and equatorial hydroxyl groups tend to react faster than axial ones [27].

In addition to the discussion above, the rotation around the C5-C6 bond in monosaccharides such as glucose, mannose and galactose is partially hindered. This gives rise to three low energy conformations also termed rotamers (gauche-gauche, gauche-trans and trans-gaucge), which are presented in the figure 14 [44]. Rotamers may affect the biological activity of the molecule as they interact with e.g. proteins [45].

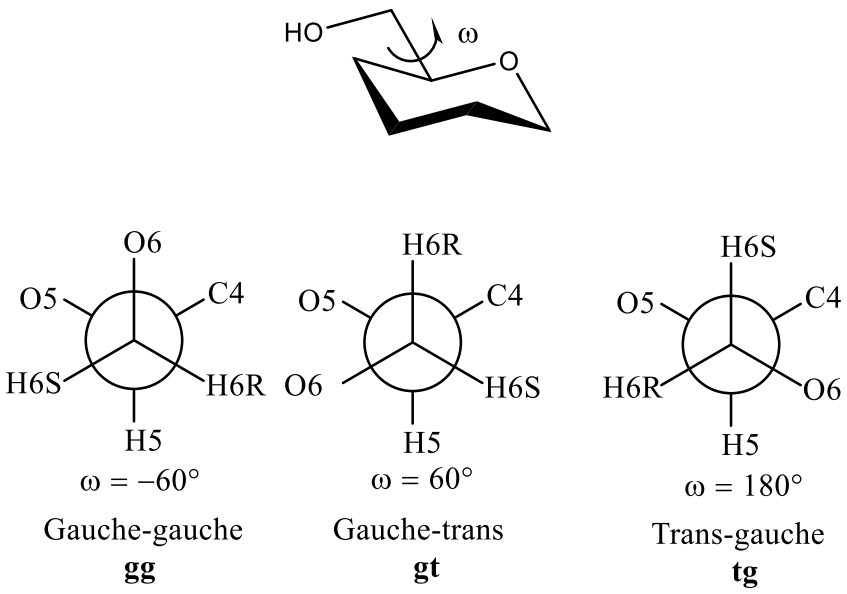


Figure 14 Rotamers of the hydroxy methylene group in the pyranose ring.

3.2 Synthetic aspects of carbohydrate chemistry

Monosaccharides such as a glucose and a galactose have primary and secondary hydroxyl groups and a hemiacetal functionality at the anomeric center. These groups have different reactivities and acidities. For example, the anomeric hemiacetal in glucose has a pKa of 12.5-14 while the hydroxyl group in position four has a pKa of 16-18. These differences can be used to achieve selectivity in synthesis [29]. Primary hydroxyl groups are sterically less hindered than secondary hydroxyl groups, they can therefore be selectively protected in the presence of the latter [26].

3.2.1 Protecting groups and strategies

As discussed above, carbohydrates contain multiple functional groups of similar reactivity and a vast amount of different protective groups and strategies have been developed to account for these facts [46]. The role of the protective groups is not limited to the masking of a hydroxyl groups, they also tune the specificity and selectivity of subsequent reactions. The most well-known examples of the dual role of protecting groups in carbohydrate chemistry is the stereoselective glycosylation reactions which will be discussed in more detail bellow [47]. Detailed reviews of protecting groups applied in carbohydrate chemistry can be found in the literature and is beyond scope of this thesis. Therefore, a brief introduction to the main protecting group types will be provided herein together with some examples from each category. At the end, the concept of orthogonal protecting group strategies will be introduced.

3.2.2 **Acetals**

Acetal protecting groups such as isopropylidene [48] and benzylidene [49] can be used to protect diols. Isopropylidene protection can be done using acetone [48] or 2,2-Dimethoxy-propane [50] under acidic conditions. A benzylidene protection can be inserted under similar reaction conditions with benzaldehyde dimethylacetal [51]. Acetals are good protecting groups as they enable the protection of 1,2-cis diols or 1,3-diols in a single step [52] [53]. The acetal groups are generally stable towards basic reaction conditions and can be removed under acidic ones [54]. Examples of isopropylidene and benzylidene protection are presented in figure 15 [27].

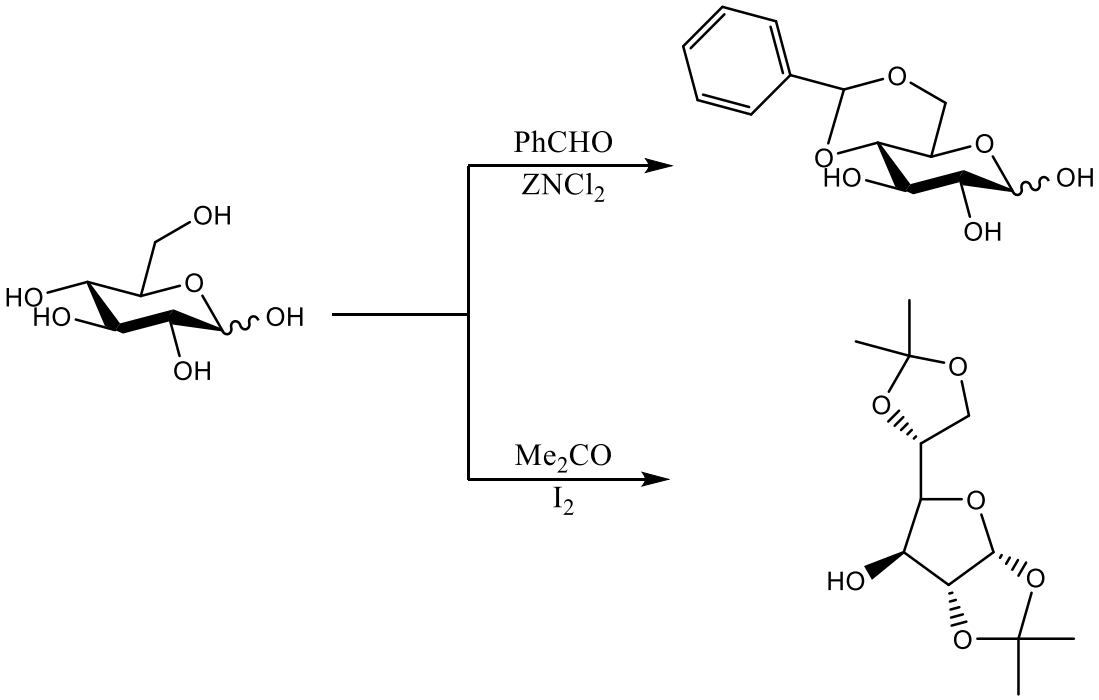


Figure 15 Regioselectivity of isopropylidene and benzylidene protection [27].

The benzylidene acetal is an especially good protective groups because It offers addition advantages over other acetals. It can be cleaved in the presence of other acid labile protective groups by hydrogenolyses or selectively ring opened in the case of the 4,6-O-benzylidene to yield either the 4-OH/6-OBn or the 4-OBn/6-OH derivatives (see figure 16) [55] [56].

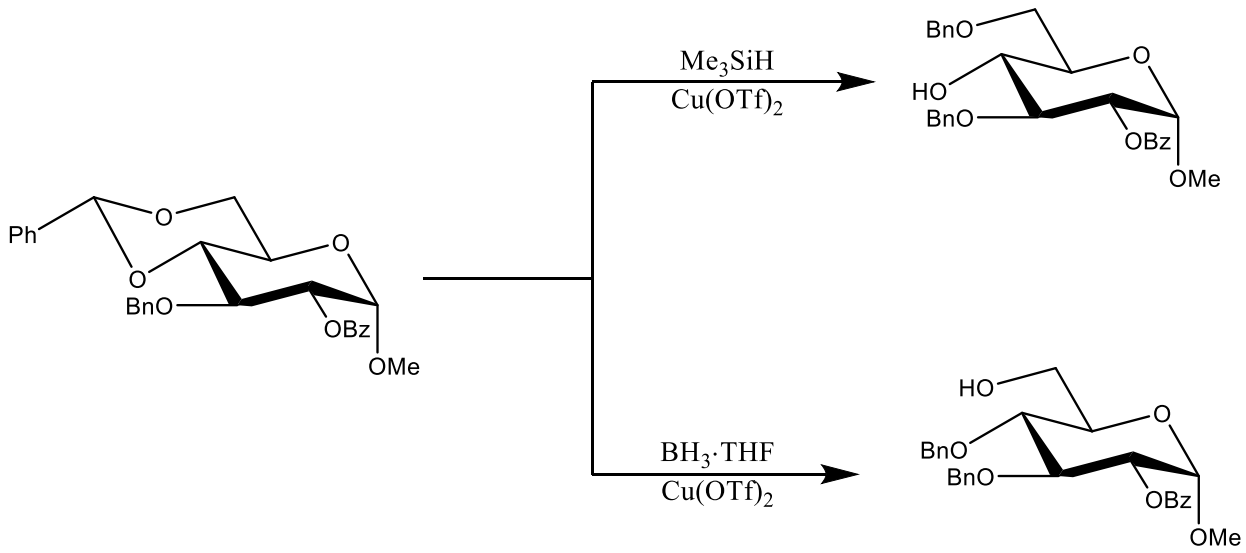


Figure 16 Regioselective ring opening of benzylidene [55] [56].

Yet another important feature of the benzylidene acetal is the conformational constraints which it adds to the parent molecule. A number of stereoselective glycosylation reactions have been deviced in which these conformational constrains play a key role in obtaining a high stereoselectivity. Examples of these include the stereoselective β-mannysylation and α-glucosylation reactions (illustrated in figure 17) [57] [58] [59].

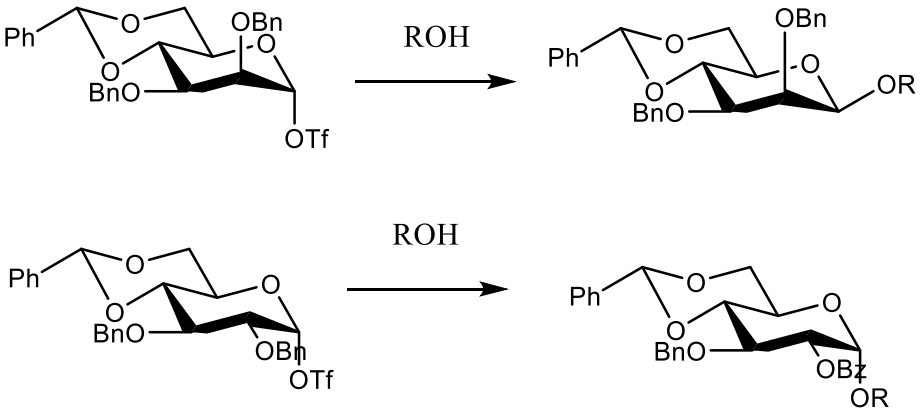


Figure 17 Glycosylation of D-mannose and D-glucose with 4,6-benzydiline protection.

3.2.3 **Ethers**

Many different kinds of ether protective groups exist. The most common ones include benzyl and silyl ethers of different types but also allyl and propargyl ethers have their merits in carbohydrate synthesis [60] [61] [62]. Benzyl ethers are inserted under basic conditions by the use of benzyl bromide and sodium hydride [60]. Milder conditions for benzylation reactions also exist. The benzyl ethers are stable towards a wide range of reaction conditions. Benzyl groups are generally deprotected by hydrogenolyses, however, the paramethoxybenzyl group can be deprotected also selectively in the presence of benzyl group e.g. under acidic conditions [63] [64].

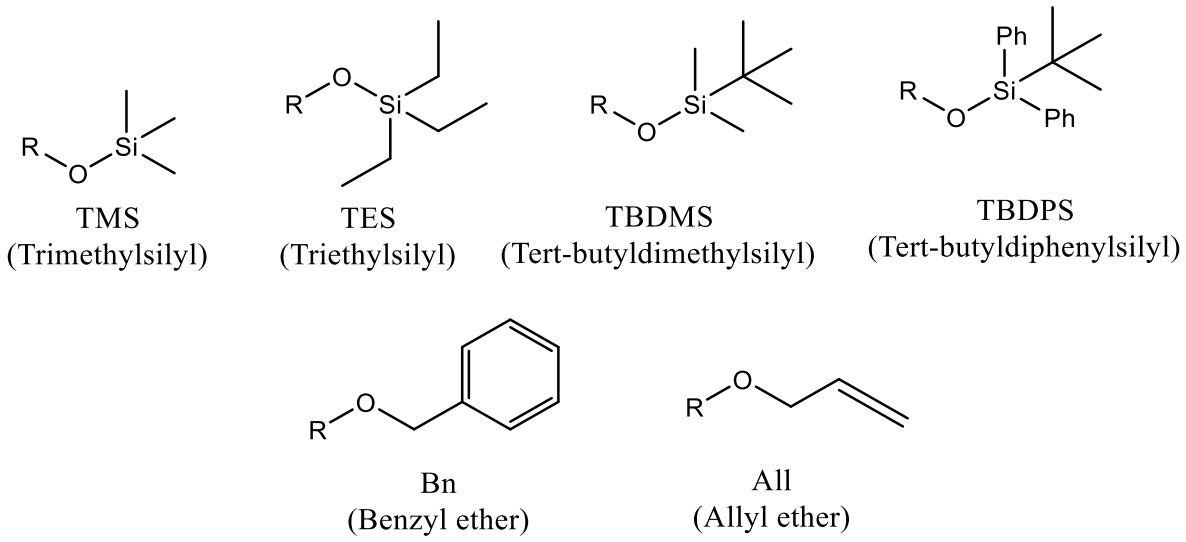


Figure 18 Some examples of ether protecting groups [66] [65] [59].

Silyl groups are widely used as protective groups in the total synthesis of natural products. They are inserted by reacting an alcohol with a silyl chloride under basic conditions. The selective protection of a primary hydroxyl group in monosaccharides can be achieved with bulky silyl chlorides such as the TBDMSCl. [65] [66].

The stabilities of silyl ethers depend on the groups attached to the silicon atom. Silyl ethers with bulky groups are more resistant to acidic and basic conditions. These intrinsic properties allow for the selective deprotection of different silyl groups within the same molecule [61] [54]. The silyl groups can be deprotected under acidic conditions and the stability order for different silyl protective groups are: TIPS>TBDMS>TES>TMS. Deprotection of silyl group can also be achieved with fluoride atom due to its high affinity for the silicon atoms [54] [67].

3.2.4 **Esters**

Ester protecting groups include acetates [68], benzoates and substituted acetates [69]. A great majority of synthetic routes in carbohydrate chemistry start from the acetylation/benzoylation of the monosaccharide in order to make them soluble in organic solvents. The esterification can be conducted under basic [68] or acidic [70] conditions or with an organometallic catalyst [71]. Acetic anhydride is a commonly employed reagent in acetylation reactions while benzoyl chloride is used in benzolytation reaction. While most reactions are performed with pyridine as a base, this generally give an anomeric mixture of peracetylated/perbenzyolated sugars. High stereoselectivity is achievable by the use of different reaction conditions (see Figure 19) [68] [72].

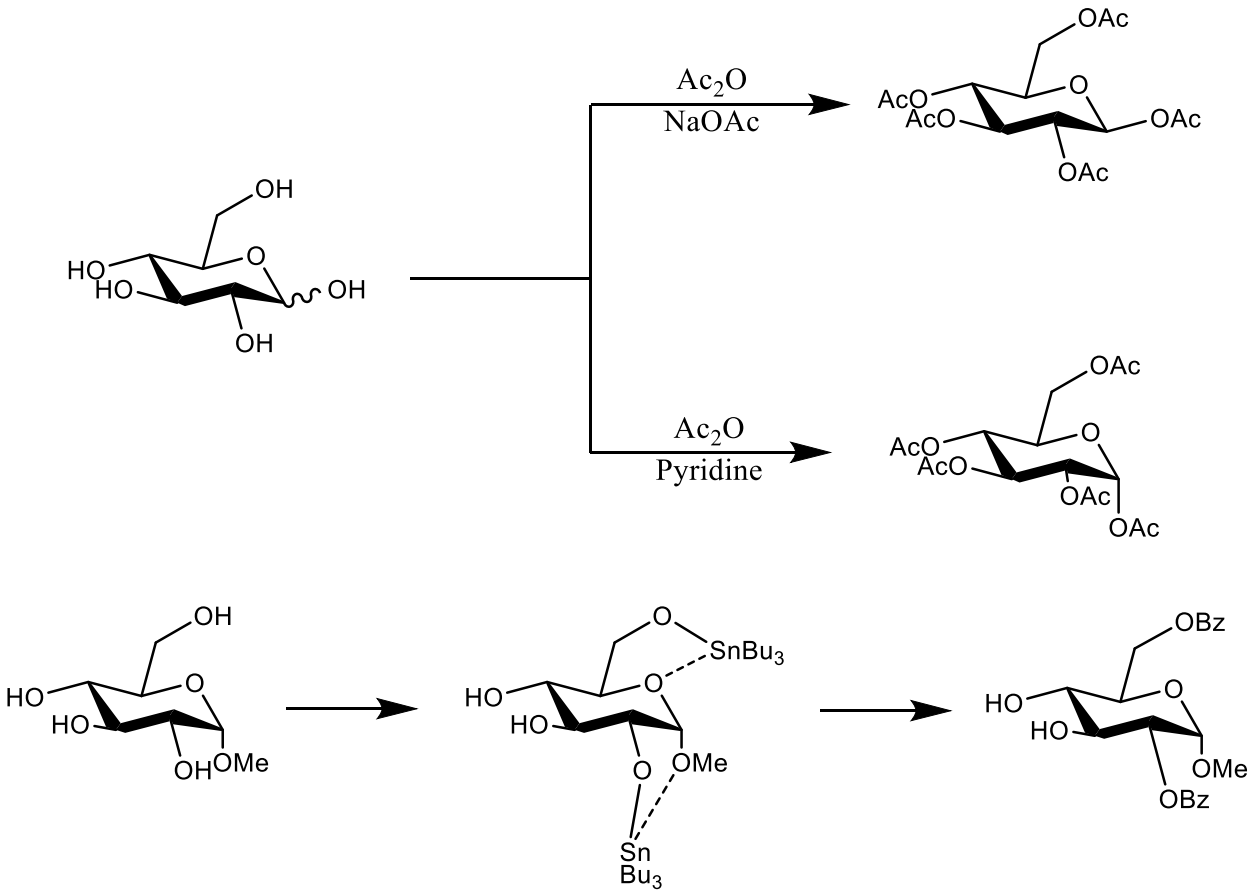


Figure 19 Selective acetylation and benzoylation reactions are displayed [68] [72].

Substituted acetates (figure 20) are modified acetates that has different reactivity compared to unmodified acetates. Addition of halides to acetate makes them more reactive towards nucleophiles as the electron density on carbonyl carbon is decreased. For example, α-chloro substituted acetate can be cleaved under mildly basic conditions in contrast to normal acetates [73]. Deprotection of esters is often conducted under basic conditions [54].

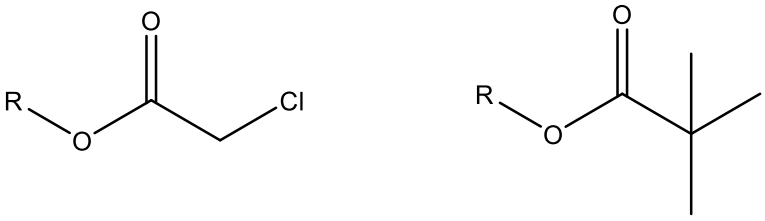


Figure 20 Examples of substituted acetates [73] [74].

3.2.5 **Carbonates**

Carbonate are used to protect diols [75] or single hydroxyl groups [76]. Their chemistry resembles that of esters to a certain degree. Reagents such as phosgenes are used to introduce carbonate protection. Cyclic 1,2- or 1,3-diol carbonate can be opened regioselectively to give the monoprotected products [77]. While carbonates can be removed under basic conditions they are not as base labile as the esters due to the stabilizing effect of the partial delocalization of the free electrons of the second oxygen [75]. Carbonates are versatile protective groups, not only can they be removed under basic conditions, other deprotection protocols varying from hydrogenolysis to photolysis can also be applied depending on the carbonate used [77]. In figure 21 examples of carbonate protected sugars are presented.

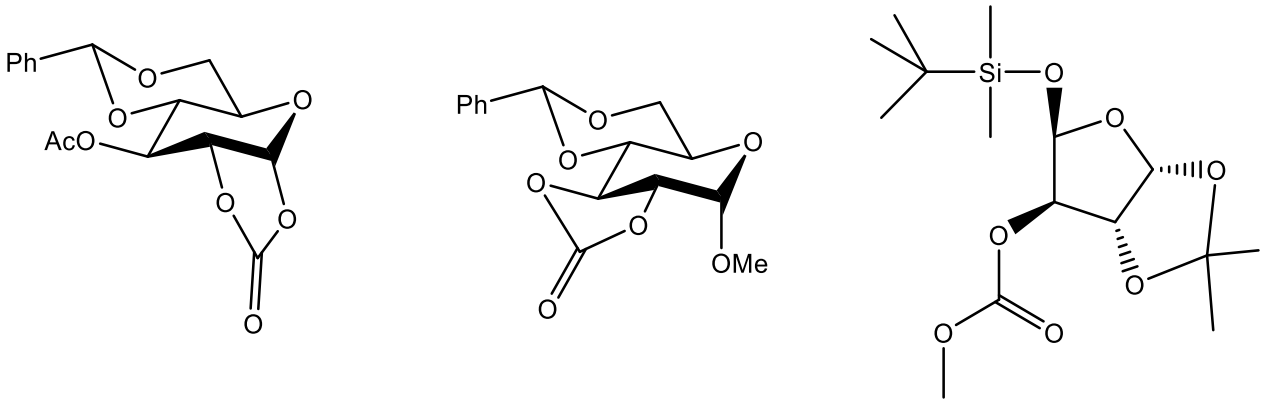


Figure 21 Examples of carbonate protected sugars.

Alkoxycarbonyl group protection is commonly done with chloroformate using mild or strong base catalyst. Mild bases like pyridine and stronger bases such sodium hydroxide are used [78]. Different side chains such as methyl, ethyl, isobutyl [75] or allyl can be used in order to fine tune the structural properties and reactivity of the carbonates (figure 22) [79].

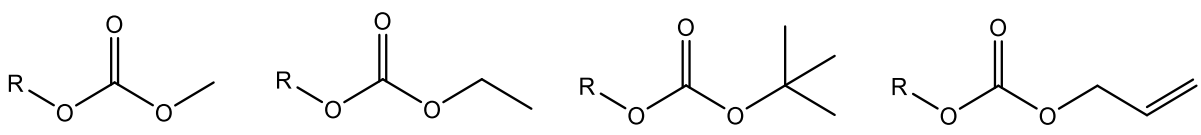


Figure 22 Examples of alkoxycarbonyl protecting groups [79].

3.2.6 **Anomeric protective groups and the glycolysation reaction**

A covalent bonding between the anomeric carbon and another molecules is called a glycosidic bond or linkage. The formation of glycosidic linkages is a key step in carbohydrate synthetic route and a trademark of oligosaccharide synthesis. Depending on the attached atoms, the sugars are called S, O, N or C glycosides (figure 23) [80] [81] [82].

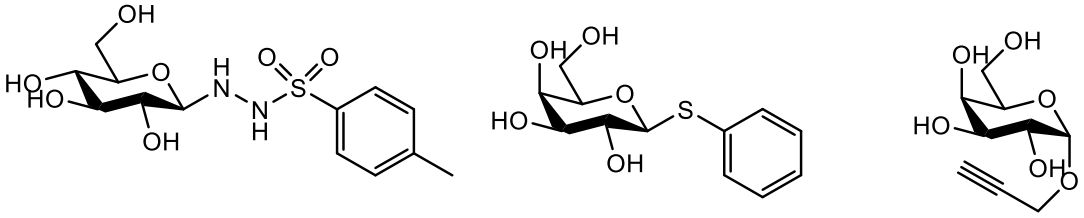
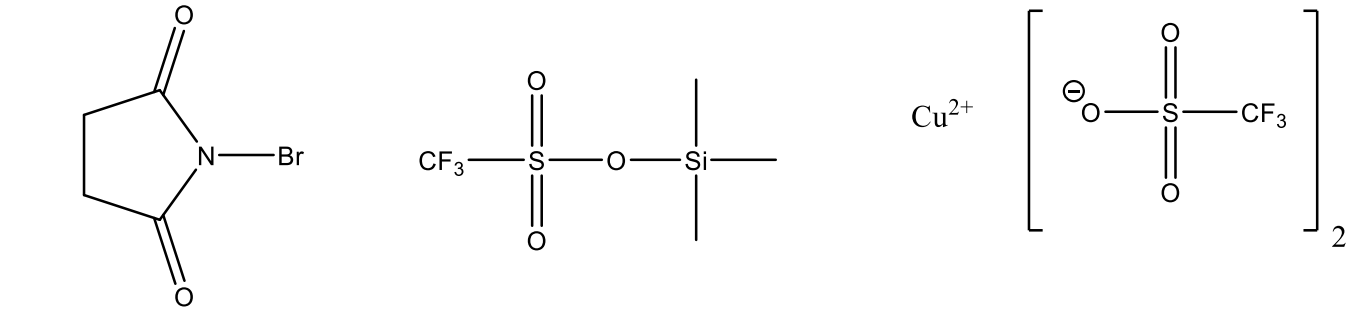


Figure 23 Examples of S-, N- and O-glycosides [83]

The anomeric centre can be protected as a glycoside thus preventing additional reactions in this position. Common anomeric protective groups are alkyl, benzyl, allyl [43] and thiols [94]. The anomeric position can be activated by acids or bases. Acid activation generates a good leaving group (water) at the anomeric center while activation by base generates a nucleophile (alkoxide ion). Under acidic conditions an acid and an alcohol/thiol is usually applied while basic conditions features a base and an alkyl halide [95].

Glycosylation reactions have three components: a glycosyl donor, a glycosyl acceptor and a promotor. A glycosyl donor is a carbohydrate containing a good leaving group at the anomeric center. The promoter is needed for activation of the leaving group in the glycosylation reaction [84]. Promoter is needed if the glycosyl donor is too stable for spontaneous glycosylation. The activation mechanisms can be divided into three different categories: Direct activation, remote activation and bidentate activation. The bidentate activation entails that the promoter determines if it is a direct or remote activation [85]. A diverse set of promoters can be used. For example, metal salts, halonium reagents, organosulfur compounds and single electron transfer reagents. More information can be found in the review article by Lian *et al*. which list 66 different promoter for alkyl/aryl thioglycosides alone [86]. Some examples of promoters are presented in figure 24 [86] [84].



N-bromosuccinimide (NBS) | Trimethylsilyl trifluoromethanesulfonate (TMSOTf) | Copper(II) trifluoromethanesulfonate ($Cu(OTf)_2$)

Figure 24 Examples of promoters used in the glycosylation [86] [84].

The reaction mechanisms of glycosylation reaction proceeds via S_N1, S_N2 or CIP (Contact Ion Pair) pathways. These pathways are affected by reaction conditions e.g. the choice of solvent and reaction temperature [87]. The optimization of reaction conditions is important in order to avoid side reactions. A change in solvent, employed reagents and glycosyl donor may have dramatic effect on the outcome of a glycosylation reaction [84]. In figure 25 two glycosylation reactions are presented. There is a considerable difference in the reaction outcome if molecular sieves are added or not [88].

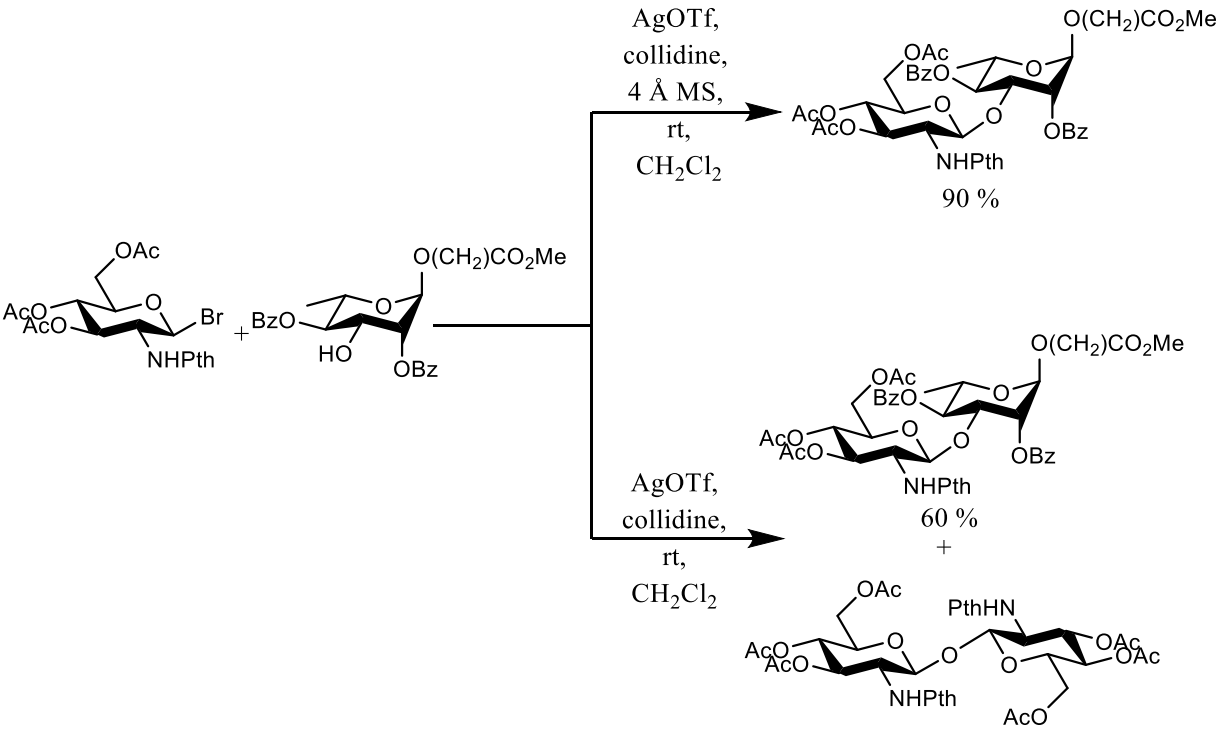


Figure 25 Example of reaction conditions effect on the outcome of glycosylation reaction [88].

Protecting groups and glycosylation reaction go hand in hand. Protecting groups can be used to influence the stereoselective outcome of glycosylation reactions. The most well-known example are the use of acyl protective groups at the position 2 in carbohydrates exemplified in Figure 26 [47]. It should also be noted that protective group free glycosylation reactions are possible [83].

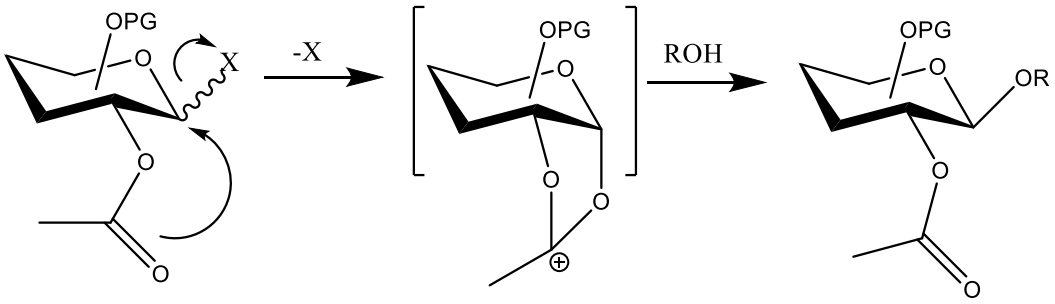


Figure 26 Participation of protecting group in glycosylation leading to β-anomer selectivity [47].

3.2.7 **Orthogonal protective group strategies**

Orthogonal protective strategies entail synthetic strategies in which different protective groups can be manipulated selectively without affecting other parts of the molecule. In carbohydrate chemistry, orthogonal protective group strategies play a key role in achieving high regioselectivities as the hydroxyl groups otherwise display similar reactivity. Above, a number of protective groups were briefly presented and focus will here be on how these groups may be combined into strategies which allow the selective manipulation of sugars in order to obtain appropriately protected building blocks for further reactions [46]. A priori when orthogonal protective group strategies are used is that the different groups have deviating stabilities under applied reaction conditions. This can be either different stabilities under similar reaction conditions as shown for the silyl ether protective groups above, or completely different protocols for their insertion/removal. Below is a schematic view of the basis for orthogonal protective groups [54]. The protective groups can me modified in order to adjust their stabilities under different reaction conditions. In multistep synthetic routes, it is often desirable that all of the protecting groups can be removed in a single step at the end of the pathway [54].

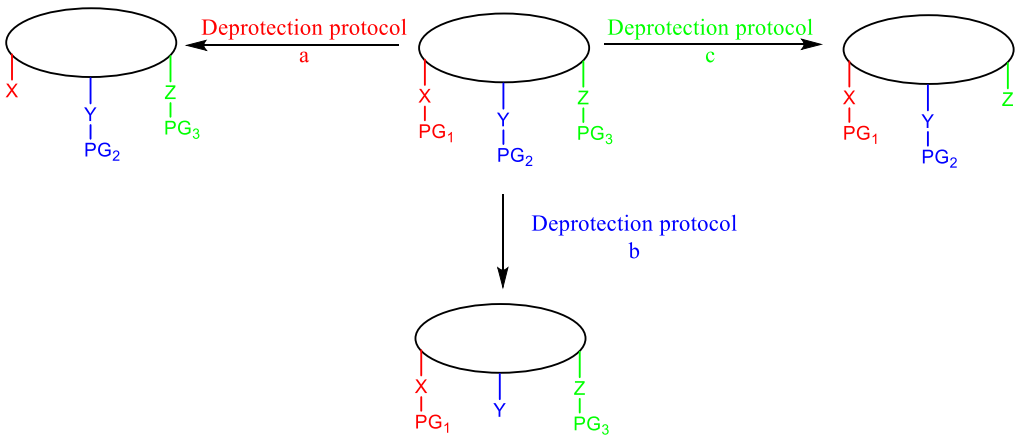


Figure 27 The concept of orthogonal protective groups is demonstrated. Different deprotection protocols lead to unmasking of selected parts of the protected molecule.

In carbohydrate chemistry, a wide variety of orthogonal protecting groups are applied. [46] [89]. For example, monosaccharides containing four hydroxyl groups and a hemiacetal may have two to five different types of protecting groups [78] [90]. Usually two temporary protecting groups are used together with some permanent protective groups. Review articles by Agoston *et al*. have summarized this topic and therefore it will not be presented here in more detail [46]. In figure 28, three different orthogonally protected sugars are displayed [78] [90].

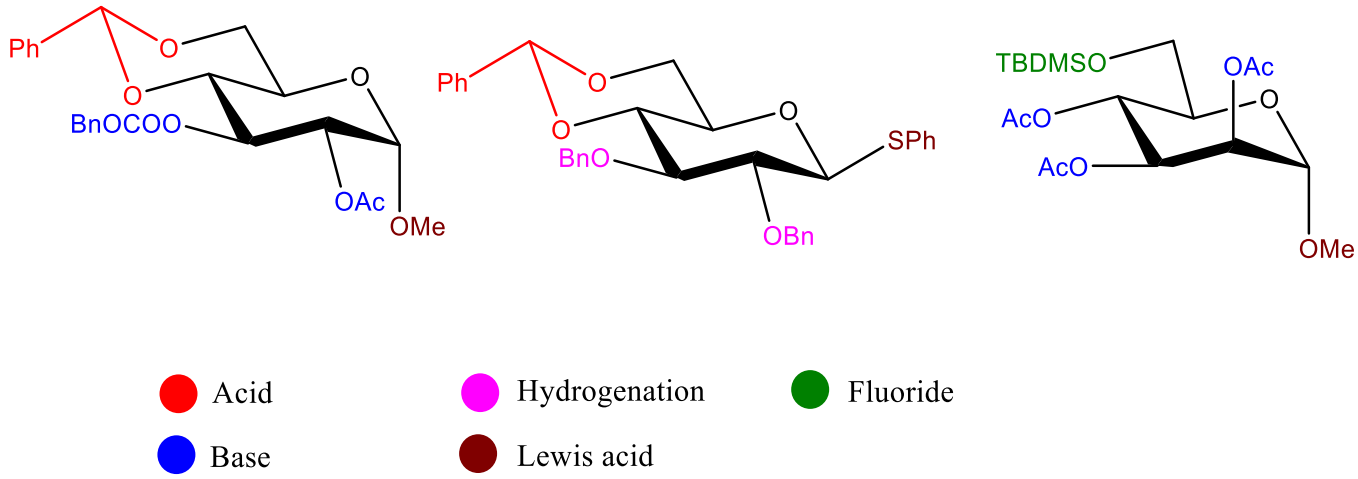


Figure 28 Orthogonally protected sugars with lability of protected groups marked [78] [90] [27].

4 AIM OF THIS STUDY

The aim of this study was to develop a synthetic route for the synthesis of D-tagaturonate. D-tagaturonate is a crucial intermediate on the D-galacturonate catabolic pathway expressed in yeast and could be used to obtain valuable insights on the enzymes functioning on this pathway (see figure 29). In addition to targeting D-tagaturonate, the synthetic pathway was designed to take into account also the production of similar compounds in order to map the structure-activity relationships and substrate scope of the corresponding enzyme on an unprecedented level.

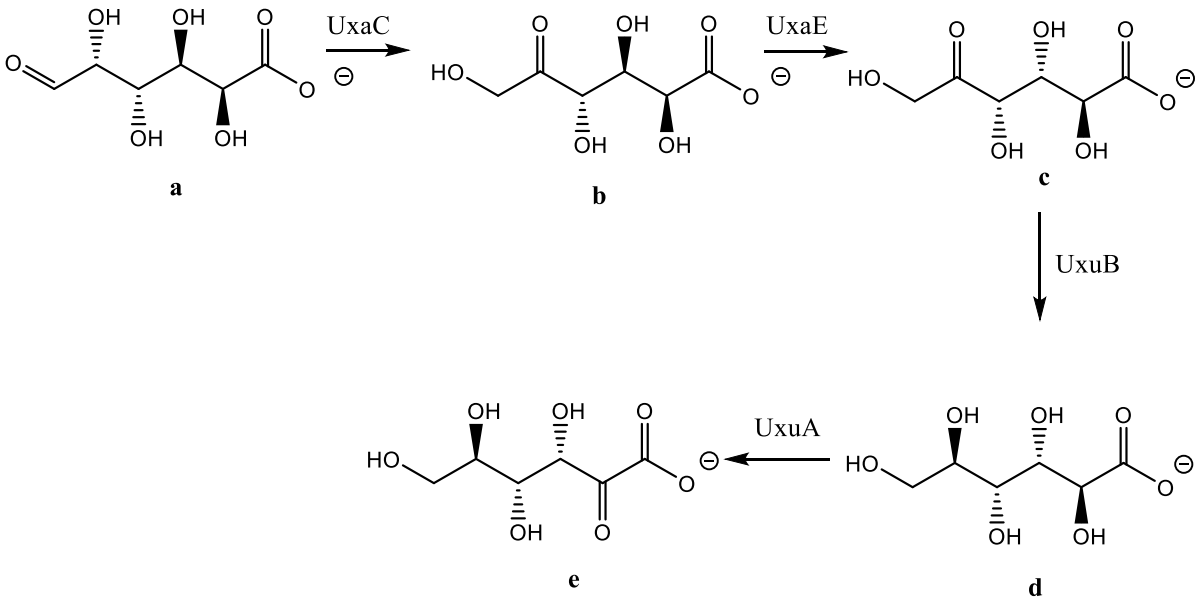


Figure 29 The catabolic pathway starts with conversion of D-galacturonate (a) to D-tagaturonate (b) by the isomerase UxaC. Epimerase UxaE converts D-tagaturonate (b) to D-fructuronate (c), which is reduced to D-mannonate (d) by UxuB dehydrogenase.

The synthetic route to D-tagaturonate can be achieved in 11 synthetic steps starting from D-galactose. This pathway includes organic transformations such as oxidation, reduction, regioselective reactions, stereoselective reactions and protective group chemistry. This challenging synthesis work together with the NMR-analytics was viewed as a good and diverse learning experience for a striving organic chemist.

5 RESULTS AND DISSCUSION

5.1 Retrosynthetic analysis

The aim of the MSc.-thesis was to develop a synthetic route for the synthesis of D-tagaturonate and its structural analogues. As can be seen from Figure 30, D-tagaturonate can be synthesized by two different routes.

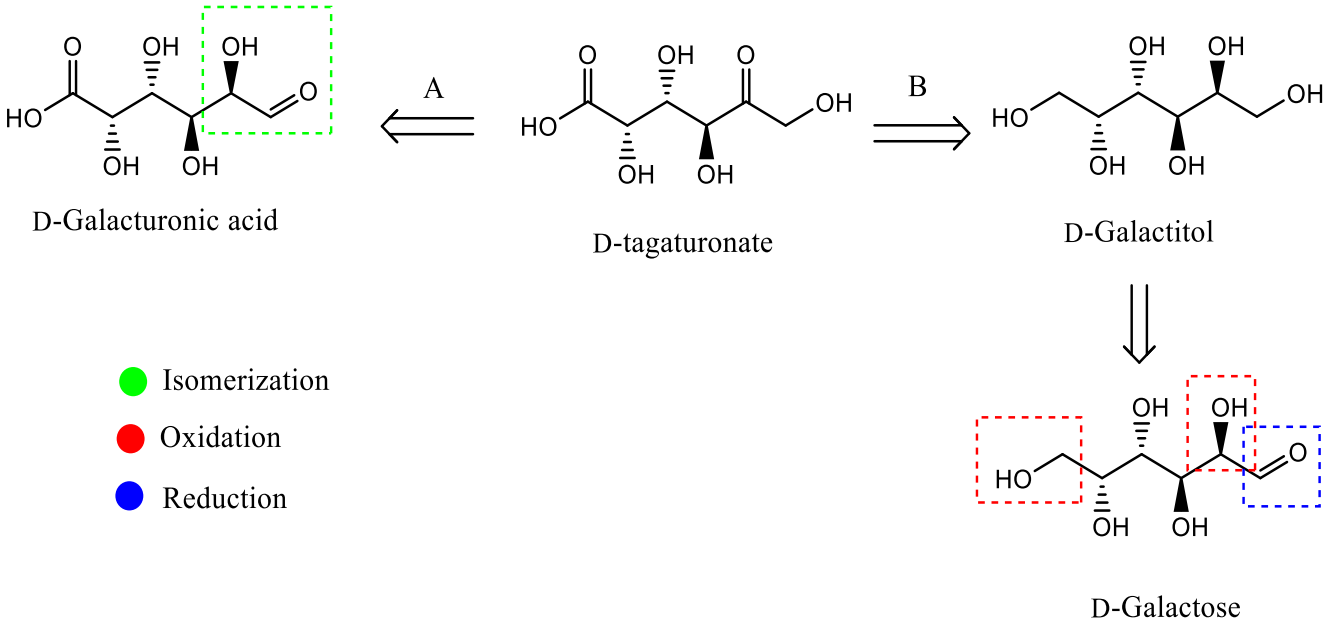


Figure 30 The retrosynthetic pathways to D-tagaturonate are displayed. Functional group interconversions are marked with colors.

The first route involves the direct synthesis of D-tagaturonate by an isomerization reaction of D-galacturonic acid. This route is the most straightforward way to the target molecule but does not offer options for further modifications to obtain different substrates.

The second strategy involves a multistep synthetic route starting from D-galactose. In order to convert D-galactose into D-tagaturonate positions 2 and 6 will have to be oxidized while position 1 will have to be reduced. This inevitably leads to a multistep synthetic strategy relying heavily upon orthogonal protecting groups. This strategy offers more options for synthesizing other structural analogies of D-tagaturonate. In order to selectively oxidize positions 2 and 6, the other positions must be protected during the oxidation step. In order to execute the needed modifications in route B, a reduction-oxidation pathway was deviced. Before the reduction, the other positions would need to be protected. After reduction, the protection of the newly formed hydroxyl groups was desirable. This would be followed by removed of the protecting groups in positions 2 and 6 and oxidation. In figure 31, the synthetic strategy is displayed.

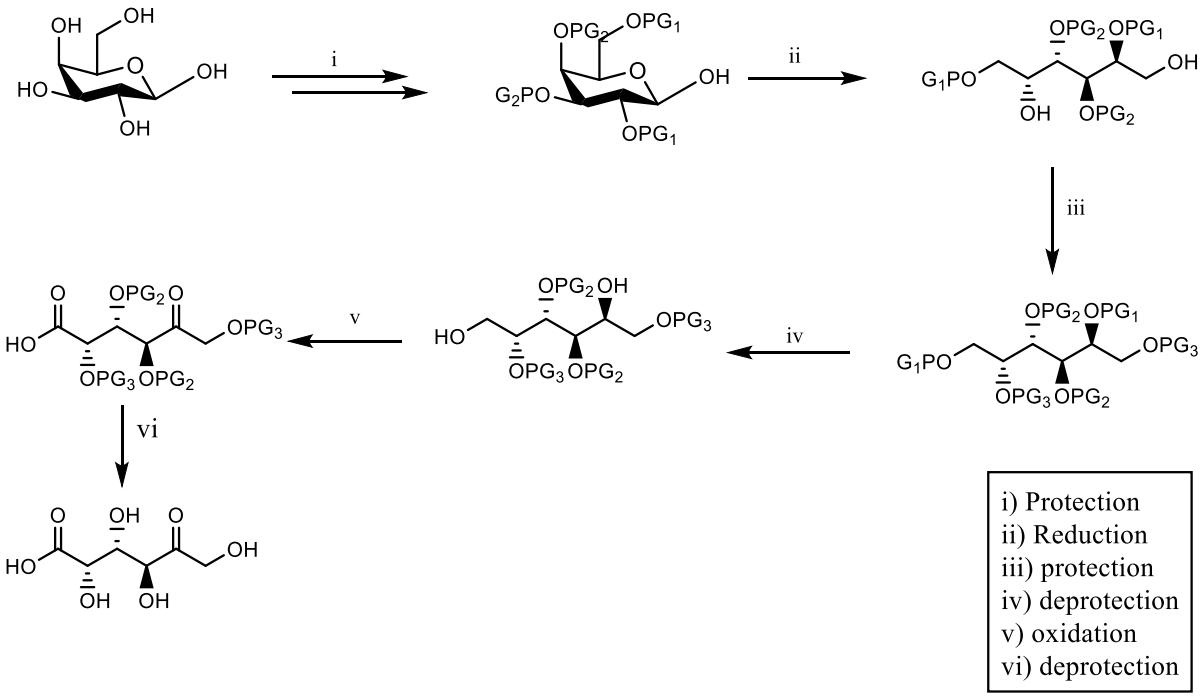


Figure 31 Synthetic strategy for route B.

5.2 Towards the synthesis of D-tagaturonate

5.2.1 Path A: Isomerization of D-galacturonic acid

In the literature, isomerization of D-galacturonic acid to D-tagaturonate has been reported under mildy basic conditions (figure 32). The reaction starts of by a CaO promoted isomerization of D-galacturonic acid in water to yield the calcium salt of D-tagaturonate. Neutralization of the salt with either oxalic acid or Dowex H^+-form would then give D-tagaturonate. We decided to start by evaluating this literature protocol. While our solution was more concentrated than in the original procedure, it did not result in the targeted product. Even following the reaction by NMR-spectroscopy did not show any indication of an isomerization taking place and this route was abandoned at an early stage. While this route was abandoned in the current work, recent publications have shown that it may have been so prematurely [91].

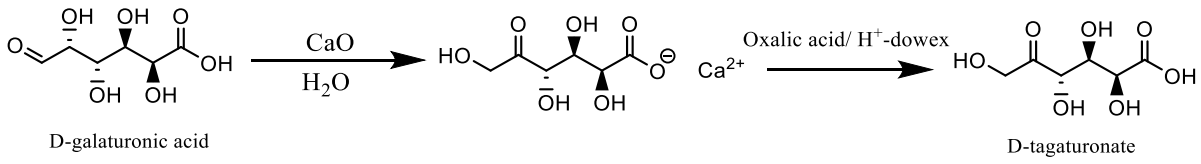


Figure 32 Isomerization of D-galacturonic acid in basic conditions.

5.2.2 **Path B: Multistep synthesis from D-galactose**

In order to solubilize D-galactose in organic solvents and to prepare a suitable glycosyl donor it was peracetylated using NaOAc/Ac_2O-protocol. This reaction gave selectively the β-anomer in a 71,2 % yield (see Figure 33). Glycosyl donor **2** was then subjected to a $BF_3{\cdot}OEt_2$ promoted glycosylation reaction with *p*-methylphenol as the nucleophile. Due to neighbouring group participation from the acetate at position 2 and the reactive nature of the nucleophile, a high β-selectivity and an excellent yield was obtained in the reaction. Next the acetyl groups were removed under Zemplen conditions [92] with NaOMe in a 1:1 mixture of the THF:MeOH thus giving 4 in a 70 % yield.

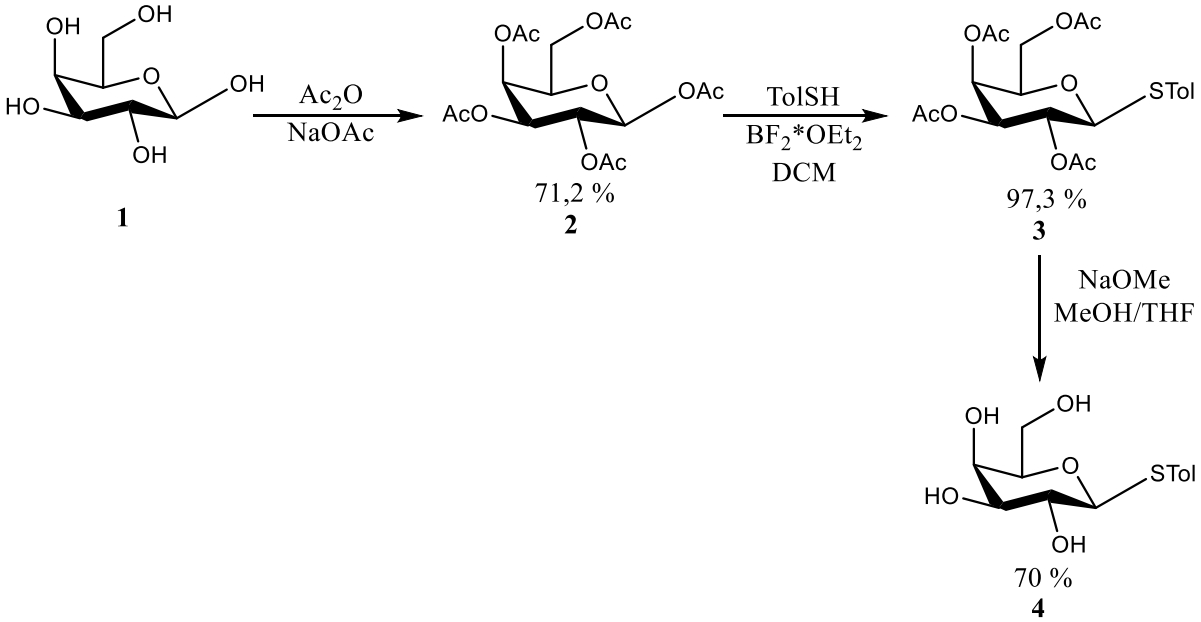


Figure 33 Synthesis of the deprotected thiol donor.

Next protection of positions 3 and 4 with an isopropylidene acetal was sought. This reaction proved more challenging than the literature indicated at a first glance. The conventional protection with 2,2-DMP (dimethylpropane) or acetone with acid catalyst such as p-TSOH (toluenesulfonic acid) or CSA (camphor sulfonic acid) gave unexpected results (see Figure 34). Instead of receiving the 3,4-*O*-isopropedyline protected galactose derivate **5**, the HMBC-spectra indicated that the isopropylidene group was attached at positions 4 and 6. This product was not received in minute quantities as it could be isolated in yields up to 84 %.

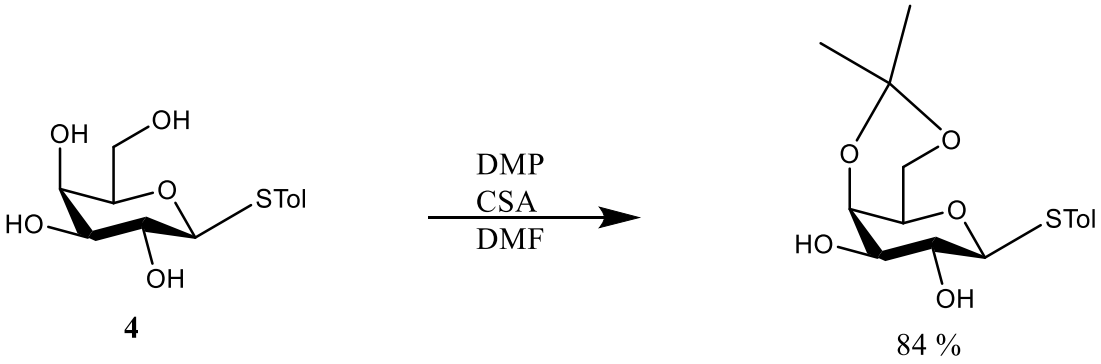


Figure 34 The unexpected outcome of isopropylidene reaction.

In order to circumvent the formation of the unexpected product, the 6th position was first modified with a bulky silyl group. Insertion of a TBDMS-ether was achieved in a yield of 87 % using TBDMSCl as the silylation reagent, imidazole as a base and DMF as the solvent. With this substrate, the instalment of the 3,4-*O*-Isopropylidene acetal worked well with previously described conditions. The removal of the silyl ether could in principle be conducted under either acidic conditions or with a fluorine source. Here, we opted to use a fluorine source as acidic conditions would be detrimental to the recently inserted 3,4-*O*-isopropylidene acetal. The silyl ether was removed in a quantitative yield with a HF-Pyridine complex in dry THF following the procedure earlier reported by Ekholm *et al* [45]. While this reaction route worked well, it added a number of reaction steps to an already lengthy reaction route and alternative conditions were sought (Figure 35).

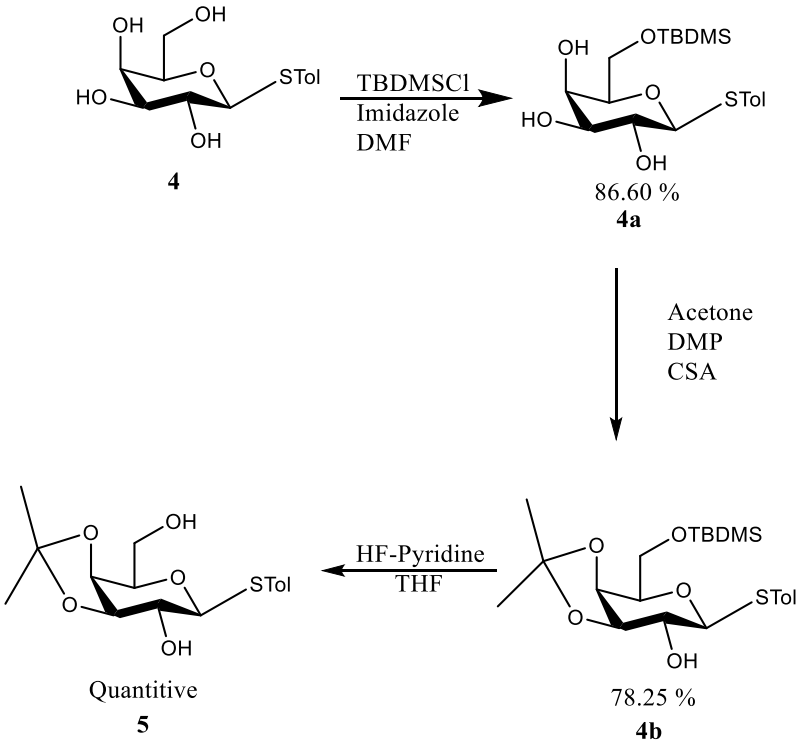


Figure 35 Alternative route to isopropydilene protection.

A direct one-pot two steps strategy was evaluated for the preparation of 6. In the first step, thiogycoside **4** was converted to the mixture of isopropylidene acetals with acetone and 2,2-

DMP under acidic conditions. After neutralization and concentration, the benzylation was conducted directly in the same pot with BnBr and NaH in dry DMF (Figure 36). The overall yield over two steps was at best 53 % although large deviations were observed thus deeming this protocol unreliable. As a result, the search for a better protocol was continued.

Figure 36 One pot reaction with isopropydilene and benzyl protection.

Similar observation as was noted in our work had also been reported previously by Catalani *et al*. [93]. To circumvent the formation of multiple isopropylidene acetals, their solution was to conduct a mild selective hydrolysis of unwanted side products to yield greater amounts of the targeted 3,4-isopropylidene derivative.

Herein, we applied a similar approach althought we opted to use PPTS (Pyridinium *p*-toluenesulfonate) as reported by McGill *et al.* [94]. With this modified protocol and alternative workup, the yield of the desired product could be increased to 97 % (Figure 37). Benzylation of the remaining two hydroxyl groups by standard protocols gave the protected galactopyranoside **6** in a 72 % yield. This two-step protocol was found to be both more efficient and reliable than the other reaction conditions tested. On a general level, it was observed that the isopropylidene group was sensitive to acidic conditions at all stages and 0.1 % Et_3N was added to the purification eluents in order to prevent cleavage during column chromatography.

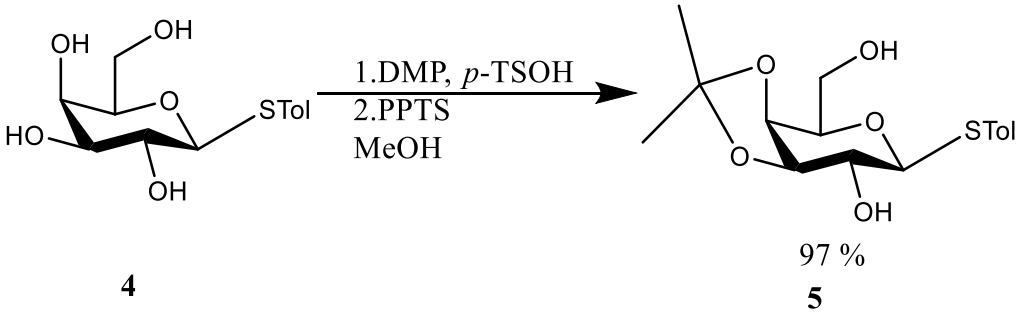


Figure 37 Optimized reaction conditions for selective insertion of a isopropylidene acetal.

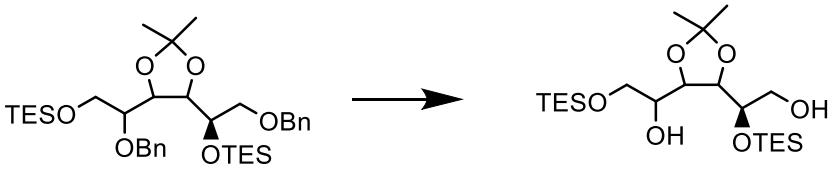
In order to reduce the anomeric position, the selective removal of thiol was required. This reaction should be viewed as a glycosylation reaction with water as a nucleophile. Owing to the nature of a thiol, the activation was attempted with the soft electrophile NBS. Originally,

the reaction mixture was brought to room temperature after the addition of NBS at 0°C, however, this resulted in cleavage of the isopropylidene group. Keeping the reaction mixture at 0°C, however, improved yields considerably (up to 70 %). With the hemiacetal obtained, the aim was to reduce it to obtain the substituted D-galactitol derivative. The reduction was carried out with $NaBH_4$ in EtOH and the desired D-galactitol derivate could be obtained in high yields.

In order to proceed on the synthetic route, the newly formed hydroxyl groups would have to be protected by a protective group that could be inserted under basic conditions and withstand the deprotection conditions of benzyl groups and tolerate oxidative reaction conditions. To this end, we chose to use a bulky silyl ether (TBDMS). The reaction proved to be difficult and only the 6-*O*-monosilylated substrate was obtained probably due to the more sterically hindered nature of the secondary hydroxyl group. By using a smaller silyl group (triethylsilyl) the protection was successful and disilylated compound was obtained in yields ranging from 70-79 %.

The next challenge awaited in the next reaction step as the deprotection of the benzyl group proved to be difficult. During normal hydrogenation conditions with Pd/C as the catalyst and MeOH, EtOH and MeOH/EtOAc as the solvent the unexpected cleavage of the silyl groups were observed. Transfer hydrogenation following the protocols of others also proved unsuccessful.

Table 2. Tested hydrogenation reactions.



Hydrogen source	Catalyst	Solvent
H_2	Pd/C	EtOAc
H_2	Pd/C	EtOAc/MeOH
H_2	Pd/C	EtOH
Cyclohexene	Pd/C	EtOH
Cyclohexene	Pd/C + 2,6-lutidine	MeOH

While examining the literature, it was noted that silyl groups can be readily cleaved under the hydrogenolysis conditions and that the choice of the solvent plays a key role on the reaction outcome. It was reported by Ikawa *et al*. [46] that solvents such as MeOH and EtOH favour desilylation while solvents such as ACN and EtOAc should not. In addition, silyl deprotection may also be the result of the partially acidic nature of the palladium catalyst [67] .

Intrigued by the possibility to obtain the desired product by a simple adjustment of the solvent, we screened the hydrogenolysis reaction in both ACN and EtOAc. In ACN, the selective cleavage of the benzyl group was successful, however, the ^{13}C- and ^{1}H-NMR spectra did not seem to be consistent with the desired product. The symmetric nature of the signal pattern observed suggest that silyl migration has taken place under these reaction conditions (^{1}H-NMR-spectra in figure 38).

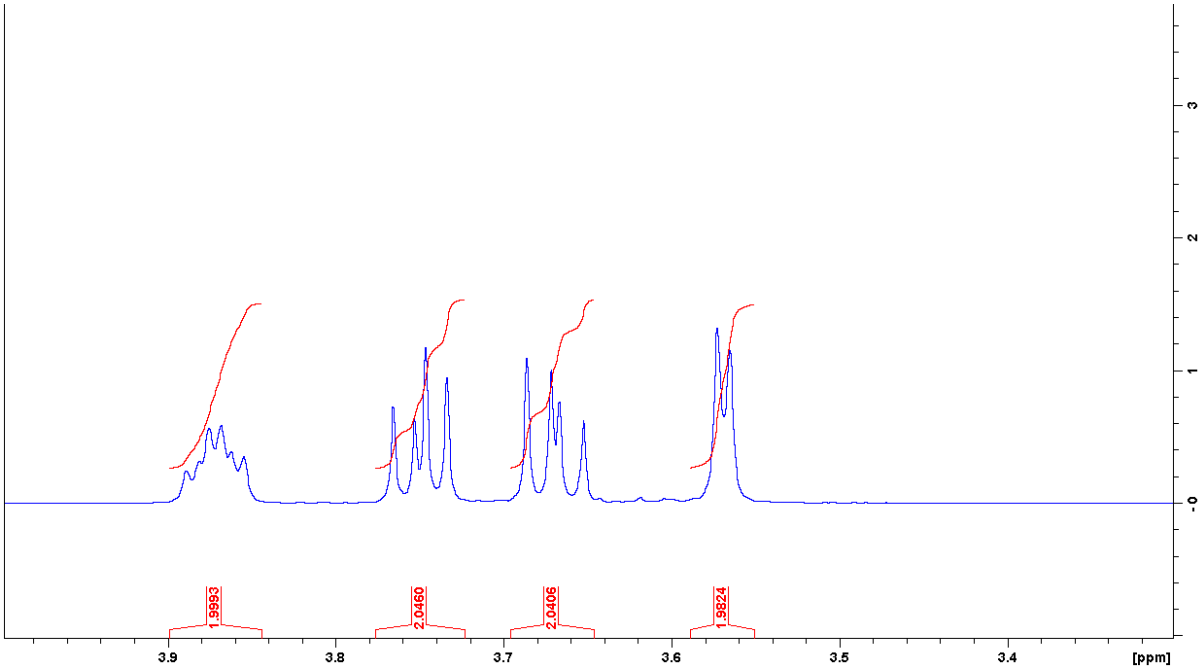
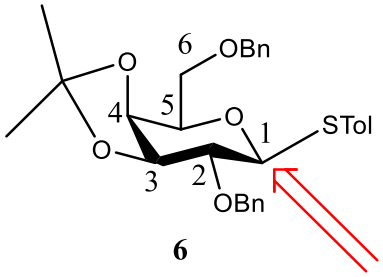


Figure 38 ^{1}H-NMR-spectra of product after catalytic hydrogenation indicates the formation of a symmetric molecule.

Due to the challenges encountered on the synthetic route and the time that was needed to resolve them, the target molecule was not reached in this MSc-thesis, however, a great deal of knowledge has been obtained which will aid in the revision of the synthetic route in future work.

5.3 NMR-spectroscopic characterization

This thesis work involved NMR-spectroscopic characterization of each product synthesized along the synthesis route. Compound **6** will serve here as an illustrative example to showcase how the NMR-spectra were interpreted. In addition to the NMR-spectroscopic analysis, mass spectrometry was utilized to confirm that the exact masses of the compounds were correct. In order to confirm that compound **6** has been synthesized, the attachment of the protective groups to the right carbon atoms must be confirmed. Along these lines, the first part of the NMR-spectroscopic characterization is to assign all signals belonging to the carbohydrate backbone. The HMBC-spectra can then be utilized to confirm the position of the protective groups. As indicated in Figure 39, the NMR-spectroscopic characterization of **6** commenced from the anomeric centre.



6

*Figure 39 The molecular structure of compound **6**. The starting point for the NMR-spectroscopic characterization is marked with a red arrow.*

The identification of H-1 in the ^{1}H-NMR spectrum was not straightforward as it overlaps with the OCH_2Ph-signals to a certain extent (figure 40). Therefore, an Ed-HSQC-spectrum was used to identify the starting point.

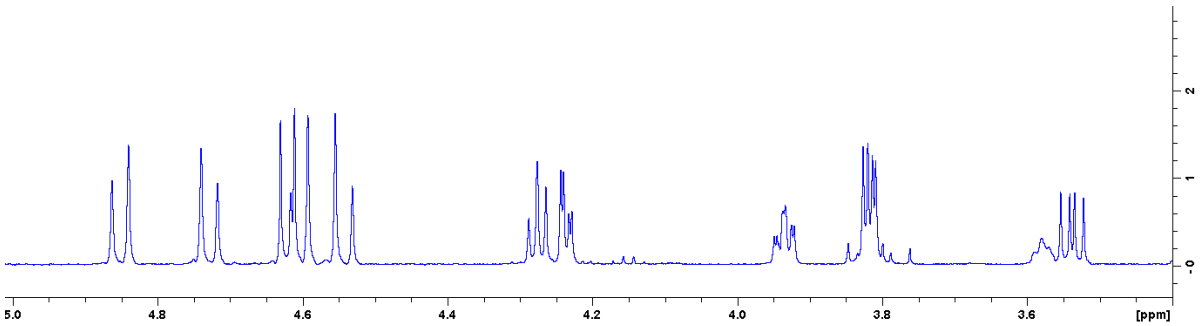


*Figure 40. The region between 5.0 – 3.5 ppm in the ^{1}H-NMR spectrum of compound **6**.*

In carbohydrates, the C-1 signal appears in a distinct area in the ^{13}C-NMR spectrum (between 110-85 ppm). In compound **6**, C-1 was identified at 86.8 ppm and H-1 as a doublet at 4.61

ppm. The large coupling constant (~7.8 Hz) observed indicated that the anomeric configuration was β. The correlation between C-1 and H-1 in Ed-HSQC-spectra of the compound **6** is presented in figure 41.

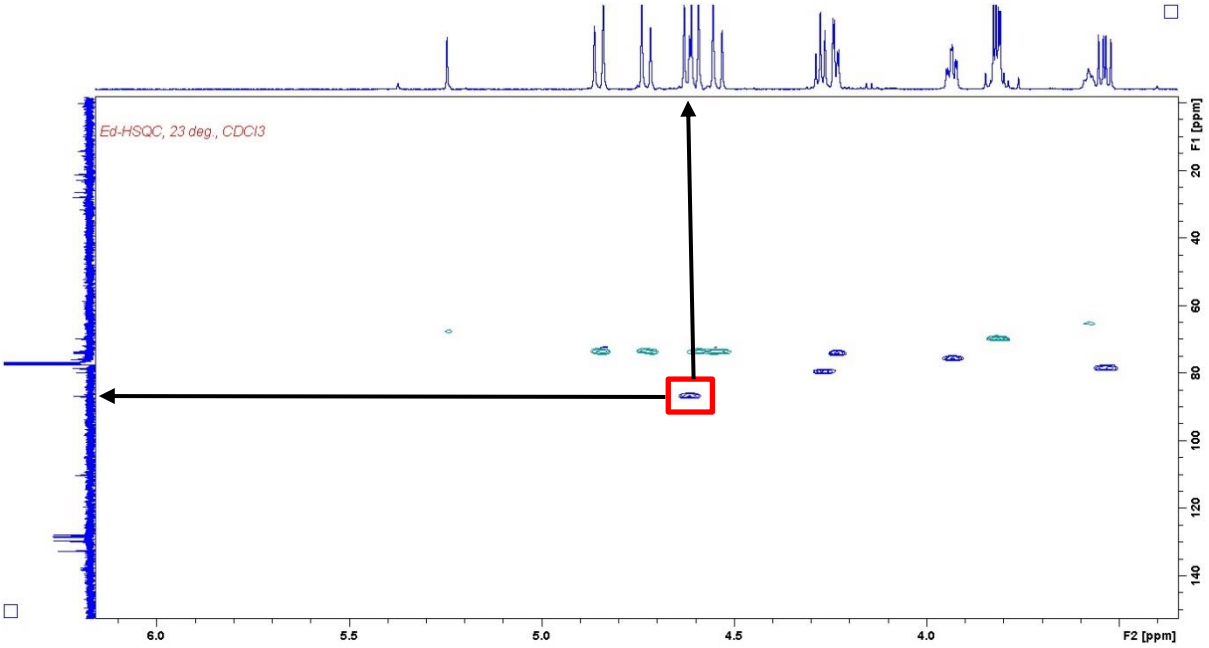


*Figure 41. HSQC-spectra of compound **6**. Presumed anomeric proton/carbon coupling marked.*

H-2 could be identified by the use of COSY which provides information on protons at neighbouring carbon atoms. A correlation between H-1 (4.61 ppm) and H-2 (3.50 ppm) is observed as indicated at figure 42 thus ensuring that these two signals are *J*-coupled to each other. By the use of Ed-HSQC, the C-2 can be identified at 78.5 ppm. By repetitive use of COSY and Ed-HSQC, all of the signals on the sugars spin system can be identified. In order to assure that that the signal assignment is logical, the individual coupling patterns can be considered (d for H-1, dd for H-2, H-3, H-4, H-6a, H-6b and ddd for H-5). Furthermore, the Ed-HSQC spectrum is able to differentiate between CH/CH_3-signals and CH_2-signals which appear in different phases. The fact that the H-6a and H-6b signals (dd at 3.78 ppm and 3.50 ppm) are in a different phase than the other signals is proof that the assignation of the carbohydrate signals are correct. With the carbohydrate signals assigned, the attention was turned to the positions of the protective groups.

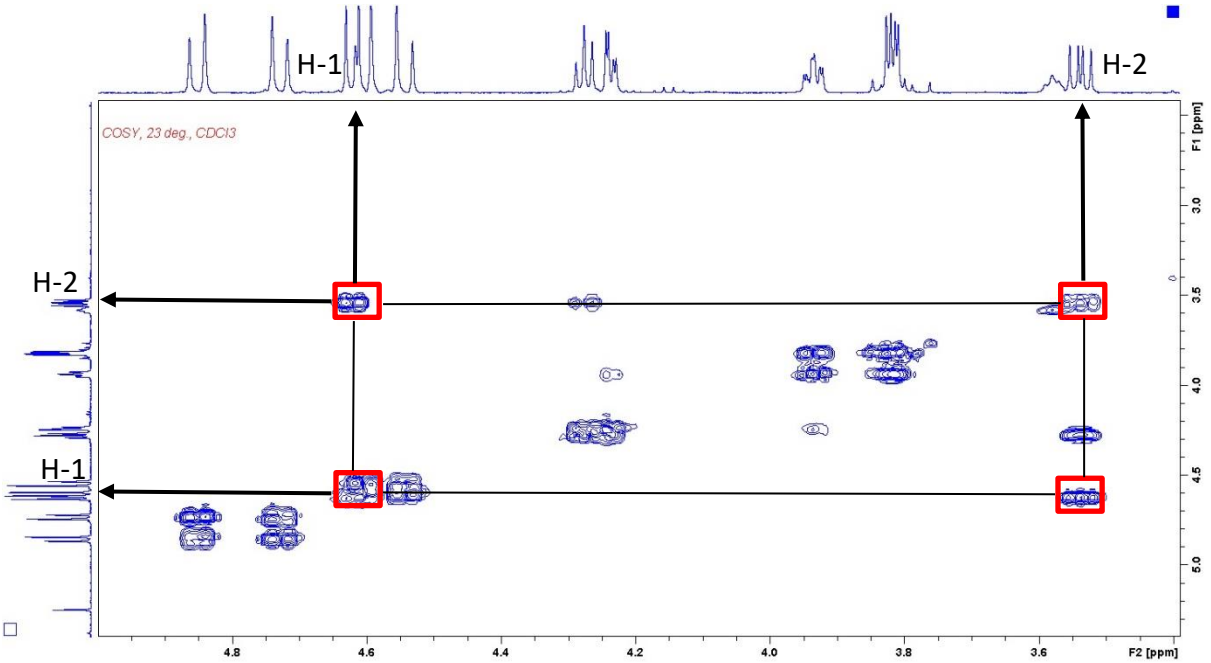


*Figure 42 The COSY spectrum of compound **6**. Correlations between H-1 and H-2 marked.*

There are two singlets in the ^{1}H-NMR-spectrum with three hydrogens at 1.33 and 1.42 ppm. These signals have a HMBC-correlation to a signal at 110.2 ppm in the ^{13}C-spectrum. These signals are characteristic for an isopropylidene acetal. The HMBC-crosspeak indicates that the isopropylidene group is attached to position 3. A HMBC correlation is not observed to position 4 but this may be due to the NMR parameters used during the measurement. Correlations of hydrogens to the carbons in the Ed-HSQC- and HMBC-spectra are presented in the figure 43.

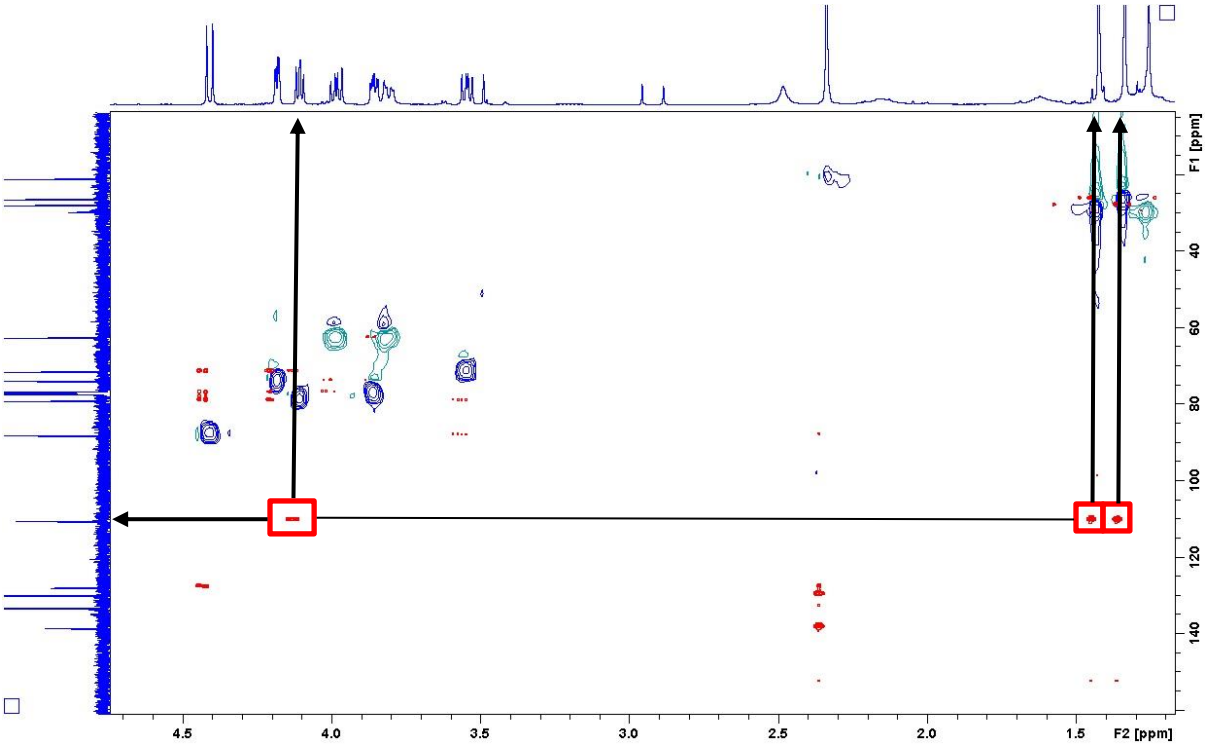


*Figure 43 HMBC- and HSQC-spectra of compound **6**. The crosspeaks of the isopropylidene group is highlighted.*

The position of the benzyl groups can be confirmed in a similar way. The HMBC correlations from the OCH_2Ph-signals (4.55 and 4.76 ppm) to carbon 4 and 6 (73.63 and 73.73 ppm) confirm that the benzyl groups are attached at the right positions. The anomeric thiophenyl group can be located in a similar way by looking at the HMBC correlation between H-1 (4.54 ppm) and the *ipso* carbon in the phenyl ring (129.7 ppm).

6 CONCLUSIONS

We set out to develop a protocol for the synthesis of the D-tagaturonate and its structural analogues. These molecules could be used to gain understanding on the enzymatic degradation pathways. D-tagaturonate is an important intermediate in the alternative D-galacturonate catabolic pathway that is not available commercially and could be used to screen the enzymatic activity on this bioengineered pathway. Insights into these pathways could lead to the better utilization of biomasses such as the pectin rich side streams of the food industry.

The synthetic route A, which had previously been reported in the literature, did not offer promising results in this thesis work. Not only did it not function well, it was also not ideal for the production of structural analogues and was therefore abandoned early on this work. Route B that was chosen as an alternative route and it proved to be filled with challenges. A great amounts of reactions were optimized during the course of this MSc-thesis work and a certain degree of work is still needed in order to reach the target molecule. Regardless, some conclusions can already be drawn. The silyl group migration witnessed in the hydrogenation step leads to the conclusion that other protective group strategies may be preferable. This will entail either changes to the silyl groups or, alternatively, the benzyl groups can be changed.

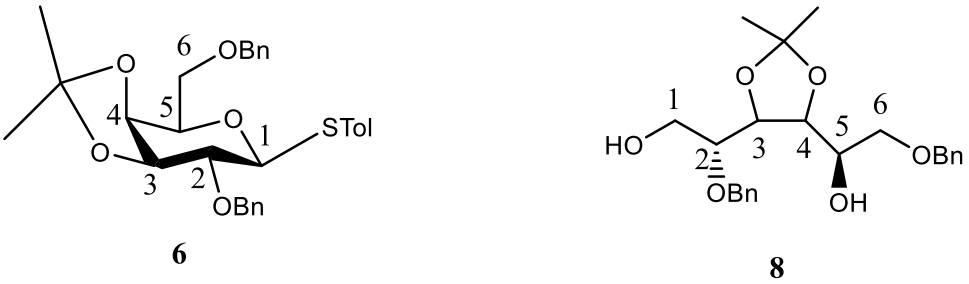
The observations done during the synthetic work are important for not only the current synthetic route but for protective group chemistry on a general level. They showcase the limitations of many of the commonly applied protective groups. These findings will therefore play a key role in the revision of the synthetic strategies which enable the synthesis of the targeted molecule and ultimately help avoid certain orthogonal protective group sets also in other complex organic synthetic work.

While in this work end product was not reached, many important observations were made following chosen synthetic route. These findings will be key importance on the future optimization of the synthetic route leading to D-tagaturonate and its structural analogues. In addition, they showcase that the traditional protecting group protocols frequently applied in the literature have their limitations when it comes to working with complex substrates such as carbohydrates. The work done in this thesis also offered significant insight into the field of organic chemistry as many different types of reactions have been performed and the product characterized in detail.

7 EXPERIMENTAL SECTION

Reaction solvents were purified with the VAC vacuum purification system prior to use when dry solvents were needed. All reactions containing moisture/air sensitive reagents were carried under an argon atmosphere. All chemicals were purchased from commercial sources. The NMR spectra were recorded with Bruker Avanche III NMR spectrometer operating at 500 MHz (^{1}H: 500.13 MHz, ^{13}C: 125.77 MHz). The probe temperature was kept at 23 °C during the experiments. The products were characterized by combination of 1D- and 2D-NMR techniques (^{1}H, ^{13}C, and COSY, ed-HSQC, HMBC, TOCSY) by using pulse sequences provided by manufacturer. Chemical shifts are expressed on the ppm scale by using TMS (tetramethylsilyl), residual chloroform or methanol as internal standard. Coupling constants are given in Hz and coupling patterns as singlet (s), doublet (d), triplet (t), multiplet (m) etc. Mass spectra were recorded with Bruker micro Q-TOF mass spectrometer with ESI (electrospray ionization) operated in positive mode. TLC was performed on aluminium sheets precoated with silica gel 60 F254 (Merck). Flash chromatography was carried out on silica gel 60 (0.040-0.060 mm, Merck). Spots were visualized by UV followed by charring with 1:10 H_2SO_4/MeOH and heating.

Numbering of the carbons in the galactitols are kept the same as in galactose (numbering presented in figure 44).



6 **8**

Figure 44 Numbering of carbons in galactose and galactitol.

7.1 Experimental procedures

1,2,3,4,6-*O*-penta-acetyl-β-D-galactopyranose (2): A suspension of acetic anhydride (50 ml, 530 mmol, 11 equiv.) and sodium acetate (3.770 g, 45.9 mmol, 1 equiv.) was heated to a boil. D-galactose (7.12 g, 45.9 mmol, 1 equiv.) was added to the resulting mixture in approximate 3 g portions. The boiling reaction mixture was heated for 1.5 h, then cooled to RT and poured onto 100 ml of an ice-water mixture. The mixture was stirred and scratched and the formed brownish solid was filtered off. The crude product was recrystallized from ethanol giving the tittle compound as white crystals (10.995 g, 71.2 %).

^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.99 (3 H, s, $COCH_3$), 2.04 (6 H, s, $COCH_3$), 2.12 (3H, s, $COCH_3$), 2.17 (3 H, s, $COCH_3$), 4.06 (1 H, dd, $J_{4,5}$ = 0.7 Hz, 7.05 Hz, H-5), 4.10-4.18 (2 H, m, H-6_a, H-6_b), 5.08 (1 H, dd, $J_{3,4}$ = 3.4 Hz, $J_{2,3}$ = 10.4 Hz, H-3), 5.33 (1 H, dd, $J_{2,1}$ = 8.3 Hz, $J_{2,3}$ = 10.4 Hz, H-2), 5.42 (1 H, dd, $J_{4,5}$ = 0.7 Hz, $J_{3,4}$ = 3.4 Hz, H-4), 5.70 (1 H, d, $J_{1,2}$ = 8.3 Hz, H-1) ppm.

^{13}C NMR (125.77 MHz, $CDCl_3$): δ 20.65-20.92 (5 C, CO**C**H_3), 61.15 (1 C, C-6), 66.91 (1 C, C-4), 67.93 (1 C, C-2), 70.95 (1 C, H-3), 71.80 (1 C, C-5), 92.26 (1 C, C-1), 169.14 (1 C, **C**OCH_3), 169.53 (1 C, **C**OCH_3), 170.12 (1 C, **C**OCH_3), 170.28 (1 C, **C**OCH_3), 170.51 (1 C, **C**OCH_3) ppm.

HRMS: m/z calcd. for $C_{16}H_{22}O_{11}Na$ [M^+Na] 413.1060, found 413.1085

***p*-Methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (3)**: To a solution containing **2** (6.2511 g, 16 mmol, 1 equiv.) in dry DCM (50 ml) was added TOLSH (3.9044 g, 31.43 mmol, 1.96 equiv.) under the argon atmosphere. The resulting mixture was cooled to 0 °C with an ice bath and $BF_3{\cdot}Et_2O$ (10 ml, 77.5 mmol, 4.84 equiv.) was added dropwise with a syringe. The mixture was brought to r.t and stirred for 2 h. The reaction was quenched by pouring it onto an aqueous $NaHCO_3$-solution and stirred for 0.5 h. The water layer was decanted off and 1 M NaOH (30 ml) was added. The mixture was stirred for another 0.5 h and the water layer was decanted off. The organic layer was washed with sat. $NaHCO_3$ (50 ml) and NaOH (50 ml). The separated aqueous layer was extracted with DCM (2x40 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to give a title compound as a white solid (7.081 g, 97.3 %).

^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.97 (3 H, s), 2.04 (3 H, s), 2.09 (3H, s), 2.11 (3H, s), 2.34 (3H, s), 3.90 (1H, t, J = 6.85 Hz), 4.09-4.02 (2H, m), 4.63-4.65 (1H, d, J 10 = Hz), 5.02-5.04 (1H, dd, J 2,7 Hz, J = 10 Hz), 5.19-5.23 (1 H, t, J = 9.75 Hz), 5.40-5.41 (1H, d, J = 3.15 Hz), 7.11-7.13 (2H, d, J = 7.85 Hz), 7.40-7.42 (2H, d, J = 7.85 Hz) ppm.

HRMS: m/z calcd. for $C_{21}H_{26}O_9SNa$ [M^+Na] 477.1195, found 477.1227

4-Methylphenyl 1-thio-β-D-galactopyranoside (4): To a solution containing **3** (16 g, 35.2 mmol, 1 equiv.) in a THF: MeOH (1:1, 100 ml) was added NaOMe (4 g, 74.03 mmol, 2.1 equiv.). The resulting mixture was stirred for 23 h and then another batch of NaOMe (3.93 g, 72.73 mmol, 2.06 equiv.) was added. After 23.5 h, the reaction mixture was neutralized by addition of Dowex 50 H^+-form. The reaction mixture was filtered and concentrated and the crude product further purified by column chromatography (DCM:MeOH 7:1) to give the title compound as a colourless oil (7.05 g, yield = 70 %).

^{1}H NMR (500.13 MHz, MeOD): δ 2.33 (3 H, s, Ph-CH_3), 3.49-3.52 (1H, dd, J 3.35 Hz, 9.2 Hz, H-3), 3.54-3.57 (1H, t, J 6.15 Hz, H-5), 3.58-3.61 (1 H, t, H-2), 3.71-3.79 (2H, m, H-6_a & H-6_b), 3.90 (1H, d, J 3.10 Hz, H-4), 4.51-4.53 (1H, d, $J_{1,2}$ = 9.68 Hz, H-1), 7.13-7.15 (2 H, d, J 8.10 Hz, arom. H), 7.47-7.48 (2 H, d, arom. H).

^{13}C NMR (125.77 MHz, MeOD): δ 21.17 (1 C, Ph-CH_3), 62.69 (1 C, C-6), 70.52 (1C, C-4), 71.13 (1 C, C-2), 76.46 (1 C, C-3), 80.68 (1 C, C-5), 90.78 (1 C, C-1), 130.62 (3 C, arom. C), 133.00 (3 C, arom. C) ppm.

HRMS: m/z calcd. for $C_{13}H_{18}O_5SNa$ [M^+Na] 309.0773, found 309.0671

4-Methylphenyl 3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (5): To a solution containing **4** (5.07 g, 177 mmol, 1 equiv.) in 2,2-DMP (50 ml) was added *p*-TsOH (0.458 g, 2.65 mmol, 0.15 equiv.) and 2,2-DMP (50 ml). The resulting mixture was stirred for 1 h 15 min. The reaction was quenched by adding 0,6 ml of Et_3N. The mixture was first concentrated and then dissolved in MeOH (120 ml). PPTS (0.51 g, 2 mmol, 0.11 equiv.) was added and the mixture was stirred for 50 min. The reaction was quenched with Et_3N and concentrated. The crude product was purified with column chromatography (DCM:MeOH 7:1) to give the product as a white foam (5.59 g, yield = 97 %).

^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.33-1.42 (6 H, 2 x s, C(C**H**$_3$)$_2$), 2.33 (3 H, s, PhC**H**$_3$), 3.54 (1 H, dd, $J_{2,3}$ = 6.99 Hz, $J_{1,2}$ = 10.28 Hz, H-2), 3.8 (1 H, dd, J, H-6$_b$), 3.84-3.86 (1 H, m, H-5), 3.98 (1 H, dd, J 7.16 Hz, 11.68 Hz, H-6$_a$), 4.10 (1 H, dd, $J_{3,4}$ = 5.53 Hz, $J_{2,3}$ = 6.99 Hz, H-3), 4.18 (1 H, dd, $J_{4,5}$ = 2.18 Hz, $J_{3,4}$ = 5.53 Hz, H-4), 4.40 (1 H, s, $J_{1,2}$ = 10.28 Hz, H-1), 7.13 (2 H, d, J = 8.03 Hz, arom. H), 7.43 (1H, d, J = 8.03 Hz, arom. H) ppm.

^{13}C NMR (125.77 MHz, $CDCl_3$): δ 26.54 (1C, C(**C**H$_3$)$_2$), 28.20 (1 C, C(**C**H$_3$)$_2$), 62.84 (1 C, C-6), 71.71 (1 C, C-2), 74.10 (1 C, C-4), 79.32 (1 C, C-3), 88.35 (1 C, C-1), 110.68 (1 C, **C**(CH$_3$)$_2$), 128.08 (1 C, arom. C), 130.05 (2 C, arom. C), 133.29 (2 C, arom. C), 138.71 (1 C, arom. C) ppm.

HRMS: m/z calcd. for $C_{16}H_{22}O_5SNa$ [M$^+$Na] 349.1086, found 349.1095

4-Methylphenyl 2,6-di-O-benzyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (6): A solution containing **5** (1.86 g, 5.69 mmol, 1 equiv.) in DMF (30 ml) was cooled to 0 °C on an ice bath and NaH (0.613 g, 25.54 mmol, 4.4 equiv.) was added. After 15 min stirring, the reaction mixture was brought to r.t and BnBr (1.8 ml, 15.15 mmol, 2.66 equiv.) was added. The reaction mixture was stirred for 17 h at r.t then cooled on an ice bath and quenched by the addition MeOH (5 ml). The reaction mixture was diluted with DCM (15 ml) and washed with brine (30 ml). The aqueous phase was extracted twice with DCM (50 + 30 ml). The combined organic layers were washed with brine (30 ml). The organic layer was dried with Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (Hex:EtOAc 4:1 + 0.1 % Et_3N) to give title compound as a white crystals (2.887 g, yield = 74 %).

^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.34 (3 H, s, C(C**H**$_3$)$_2$), 1.40 (3 H, s, C(C**H**$_3$)$_2$), 2.28 (3 H, s, PhC**H**$_3$) 3.49-3.52 (1 H, dd, $J_{1,2}$ = 9.64 Hz, $J_{2,3}$ = 6.24Hz, H-2), 3.78-3.79 (2 H, m, H-6$_{a\&b}$), 3.89-3.92 (1H, m, $J_{4,5}$ = 2.0 Hz, H-5), 4.20-4.21 (1 H, dd, $J_{4,5}$ = 2.0 Hz, H-4), 4.23-4.25 (1 H, t, $J_{2,3}$ = 6.24Hz, H-3), 4.50-4.60 (2 H, dd, C**H**$_2$-Ph), 4.58-4.50 (1H, $J_{1,2}$ = 9.64 Hz, H-1) 4.68-4.83 (2 H, m, C**H**$_2$-Ph), 7.01-7.03 (2H, d, arom. H), 7.25-7.35 (12 H, m, arom. H), 7.42-7.46 (4 H, m, arom. H) ppm.

^{13}C NMR (125.77 MHz, $CDCl_3$): δ 26.54 (1 C, C(**C**H$_3$)$_2$), 28.05 (1 C, C(**C**H$_3$)$_2$), 69.92 (1 C, C-6), 73.63 (1 C, **C**H$_2$-Ph), 73.73 (1 C, **C**H$_2$-Ph), 74.09 (1 C), 75.94 (1 C, C-5), 78.55 (1 C, C-2), 79.86 (1 C, C-3), 86.85 (1 C, C-1), 110.25 (1 C, **C**(CH$_3$)$_2$), 127.79-127.92 (3 C, arom. C), 128.44-128.53 (3 C, arom. C), 129.73 (1 C, arom. C), δ 132.69 (1 C, arom. C) ppm.

HRMS: m/z calcd. for $C_{30}H_{34}O_5SNa$ [M$^+$Na] 529.2025, found 529.2083

2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-D-galactopyranoside (7): A solution containing **6** (0.457 g, 0.9 mmol, 1 equiv.) in H_2O/Acetone (10 ml, 1:10) was cooled to 0 °C on an ice bath. To the cooled mixture was added NBS (0.303 g, 1.7 mmol, 1.88 equiv.). The mixture was brought to r.t, stirred for 21 min and quenched by addition of sat. $NaHCO_3$ (2 ml). The resulting mixture was diluted with DCM (40 ml) and washed with brine (40 ml). The aqueous phase was extracted twice with DCM (20 ml each). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (Hex: EtOAc: Et_3N 1:1:0.01) to give the title compounds as brownish crystals (0.254 g, yield = 70.27 %).

^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.31 (3 H, 1 s), 1.39 (3 H, s), 3.42-3.25 (1 H, t), 3.58-3.60 (2 H, q), 3.66-3.67 (1 H, d), 3.69-3.75 (2 H, m), 4.00-4.03 (1 H, m), 4.15-4.17 (1 H, dd), 4.21.-4.24 (1 H, t), 4.37-4.72 (2 H, m), 4.50-4.53 (1 H, d), 4.62-4.69 (2 H, m), 4.74-4.79 (2 H, m), 5.20-5.21 (1 H, m), 7.25-7.38 (20 H, m) ppm.

^{13}C NMR (125.77 MHz, $CDCl_3$): δ 22.68 (1 C), 25.96 (1 C), 27.54 (1 C), 31.61 (1 C), 67.33 (1 C), 69.61 (1 C), 71.59 (1 C), 72.78 (1 C), 73.11 (1 C), 73.29 (1 C), 73.50 (1 C), 73.60 (1 C), 73.78 (1 C), 74.60 (1 C),75.76 (1 C), 77.80 (1 C), 79.29 (1 C), 90.78 (1 C), 96.00 (1 C), 109.51 (1 C), 110.04 (1 C), 127.68-128.49 (18 C), 137.65-138.08 (4 C) ppm.

HRMS: m/z calcd. for $C_{23}H_{28}O_6Na$ [M^+Na] 423.1784, found 423.1819

2,6-di-O-benzyl-3,4-*O*-isopropylidene-D-galactitol (8): To a solution containing **7** (0.1 g, 0.22 mmol, 1 equiv.) in EtOH (20 ml) was added $NaBH_4$ (0.105 g, 2.7 mmol, 12 equiv.). The mixture was stirred overnight and then quenched with AcOH (1 ml). The mixture was diluted with DCM (20 ml) and washed with brine (20 ml). The aqueous phase was extracted with DCM (2x20 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to give the crude product. The crude product was purified by column chromatography (Hex:EtOAc 1:1) to give the title compound as a clear syrup (0.075 g, yield = 74.85 %).

1H NMR (500.13 MHz, $CDCl_3$): δ 1.37 (3 H, C(C**H**$_3$)$_2$), 1.53 (3 H, C(C**H**$_3$)$_2$), 3.51-3.56 (2 H, m), 3.69-3.72 (1H, dd), 3.80-3.82 (1H, q), 3.84-3.87 (1H, m), 3.89-3.92 (1H, m), 4.21-4.22 (1H, dd), 4.34-4.37 (1 H, dd), 4.53 (2 H, s), 4.66-4.75 (2 H, m), 7.25-7.38 (10 H, m, arom. H) ppm.

13C NMR (125.77 MHz, $CDCl_3$): δ 14.29 (1 C), 22.82 (1 C), 25.52 (2 C), 26.49 (2 C), 31.75 (1 C), 62.64 (1 C), 68.65 (1 C), 71.55 (1 C), 72.38 (1 C), 73.62 (1 C), 76.32 (1 C), 76.76 (1 C), 78.49 (1 C), 109.04 (1 C), 127.90 (1 C, arom. C), 127.94 (2 C, arom. C), 128.21 (1 C, arom. C), 128.41 (2 C, arom. C), 128.57 (2 C, arom. C), 128.70 (2 C, arom. C), 137.56 (1 C, arom. C), 138.21 (1 C, arom. C) ppm.

HRMS: m/z calcd. for $C_{23}H_{30}O_6SNa$ [M^+Na] 425.1940, found 425.1986

2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-1,5-di-*O*-triethylsilyl-D-galactitol (9): A solution containing **8** (0.106 g, 0.26 mmol, 1 equiv.) in pyridine (5 ml) was cooled to 0 °C on an ice bath. To the cooled solution was added TESCl (0.1 ml, 0.738 mmol, 2.8 equiv.). The mixture was brought to r.t and stirred for 1 h. The reaction was quenched with H_2O (5 ml) and diluted with DCM (30 ml). The mixture was washed with brine (20 ml) and aqueous phase was extracted with DCM (20 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (Hex:EtOAc 4:1 + 0.1 % Et3N) to give the title compound as clear syrup (0.132 g, 79.27 %).

1H NMR (500.13 MHz, $CDCl_3$): δ 0.49-0.61 (18 H, m, TES H), 0.87-0.95 (12 H, m, TES H), 1.33 (3 H, s, C(C**H**$_3$)$_2$), 1.50 (3 H, s, C(C**H**$_3$)$_2$), 3.45-3.51 (2H, m), 3.67-3.71 (1H, m), 3.77-3.82 (2 H, m), 4.11-4.14 (2 H, m), 4.50 (2H, s), 4.70-4-75 (2 H, m), 7.22-7.37 (10 H, m, arom. H) ppm.

13C NMR (125.77 MHz, $CDCl_3$): δ 4.46 (3 C), 5.40 (3 C), 6.60 (1 C), 6.95 (3 C), 7.08 (3 C), 25.97 (1 C), 26.59 (1 C), 63.77 (1 C), 71.27 (1 C), 72.82 (1 C), 73.26 (1 C), 73.53 (1 C), 77.80 (1 C), 79.14 (1 C), 108.44 (1 C), 127.34 (1 C), 127.54 (2 C), 127.69 (1 C), 128.28 (2 C), 128.45 (2 C), 138.45 (1 C), 139.36 (1 C) ppm.

HRMS: calc. for $C_{35}H_{58}O_6Si_2Na$ 653.3670, found 653.3678

4-Methylphenyl 6-*O*-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside (4a): A solution containing **3** (0.985 g, 3.43 mmol, 1 equiv.) and imidazole (0.5 g, 7.34 mmol, 2.13 equiv.) in DMF was cooled to 0 °C on an ice bath. To the cooled solution was added TBDMSCl (0.803 g, 5.32 mmol, 1.53 equiv.) was added. The solution was brought to r.t and left to stir overnight. After 24 hours, the solution was cooled on an ice bath and additional Imidazole (0.363 g, 5.33 mmol, 1.55 equiv.) and TBDMSCl (0.893 g, 5.92 mmol, 1.72 equiv.) was added. After 2 h, the reaction mixture was concentrated. The crude product was purified by column chromatography (DCM:MeOH 15:1 → DCM:MeOH 5:1) to give the title compound as a white powder (1.19 g, yield = 86.60 %).

^{1}H NMR (500.13 MHz, $CDCl_3$): δ 0.09 (3 H, s, Si(C**H**$_3$)$_2$), 0.10 (3 H, s, Si(C**H**$_3$)$_2$), 0.90 (9 H, s, SiC(C**H**$_3$)$_3$), 3.50-3.52 (1 H, m, H-5), 3.57-3.59 (1 H, dd, H-3), 3.63-3.66 (1 H, t, H-2), 3.87-3.96 (2 H, m, H-6$_{a\&b}$), 4.07-4.08 (1 H, d, H-4), 4.45-4.46 (1 H, d, H-1), 7.10-7.11 (2 H, d, arom. H), 7.45-7.46 (2 H, d, arom. H) ppm.

^{13}C NMR (125.77 MHz, $CDCl_3$): δ -5.30—5.28 (2 C), 25.98 (3 C), 63.29 (1 C, C-6), 69.45 (1 C, C-4), 70.05 (1 C, C-2), 75.19 (1 C, C-3), 78.27 (1 C, C-5), 88.92 (1 C, C-1), 128.64 (1 C, arom. C), 129.88 (2 C, arom. C), 133.15 (2 C, arom. C), 135.24 (1 C, arom. C) ppm.

4-Methylphenyl 3,4-*O*-isopropylidene-6-*O*-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside (4b): To solution containing **4a** (1.19 g, 2.72 mmol, 1 equiv.), 30 ml of DMF and 12 ml of DMP (10.18 g, 97.79 mmol, 35.95 equiv.) was added CSA (0.095 g, 0.4 mmol, 0.14 equiv.). The reaction was left to stir overnight. The reaction was neutralized with Et_3N and concentrated. The crude product was purified by column chromatography (DCM:MeOH 15:1 + 0.1 % Et_3N) to give the title compound as a white solids (0.938 g, yield = 78.25 %).

^{1}H NMR (500.13 MHz, $CDCl_3$): δ 0.08 (6 H, s, Si(C**H**$_3$)$_2$), 0.90 (9 H, s, SiC(C**H**$_3$)$_3$), 1.32 (3 H, s, C(C**H**$_3$)$_2$), 1.43 (3 H, s, C(C**H**$_3$)$_2$), 2.33 (3 H, s, Ph-C**H**$_3$), 3.52-3.56 (1 H, m, $J_{1,2}$ = 10.28, H-2), 3.78-3.81 (1 H, m, H-5), 3.87-3.88 (1 H, d, H-6$_{a\&b}$), 4.04-4.06 (1 H, m, H-3), 4.21-4.23 (1 h, dd, H-4), 4.37-4.39 (1 H, d, $J_{1,2}$ = 10.28 Hz, H-1), 7.10-7.11 (2 H, d, arom. H), 7.42-7.44 (2 H, d, arom. H) ppm.

^{13}C NMR (125.77 MHz, $CDCl_3$): δ 26.02 (3 C, SiC(**C**H$_3$)$_3$), 26.49 (1 C, Si(**C**H$_3$)$_2$), 28.33 (1 C, Si(**C**H$_3$)$_2$), 62.37 (1 C, C-6), 71.91 (1 C, C-2), 73.36 (1 C, C-4), 79.15 (1 C, C-3), 88.91 (1 C, C-1), 110.21 (1 C, **C**(CH$_3$)$_2$), 128.83 (1 C, arom. C), 129.95 (2 C, arom. C), 133.02 (2 C, arom. C), 138.38 (1 C, arom. C) ppm.

4-Methylphenyl 3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (5): Solution of **4b** (0.142 g, 0.322 mmol, 1 equiv.) in THF (3 ml) was cooled to 0 °C on an ice bath. To the solution was added 150 µl of HF·pyridine (5.77 mmol, 17.92 equiv.). The mixture was brought to room temperature and left to stir overnight. The reaction mixture was diluted with DCM (5 ml) and quenched by addition of sat. $NaHCO_3$ (12 ml). The organic phase was washed with brine (10 ml) and the aqueous phase extracted with DCM (10 ml). The separated water layer was extracted with 10 ml of DCM. The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (DCM:MeOH 7:1) to give the title compound as a white foam (0.116 g, 100 %).

^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.33-1.42 (6 H, 2 x s, C(C**H**$_3$)$_2$), 2.33 (3 H, s, PhC**H**$_3$), 3.54 (1 H, dd, $J_{2,3}$ = 6.99 Hz, $J_{1,2}$ = 10.28 Hz, H-2), 3.8 (1 H, dd, J, H-6$_b$), 3.84-3.86 (1 H, m, H-5), 3.98 (1 H, dd, J 7.16 Hz, 11.68 Hz, H-6$_a$), 4.10 (1 H, dd, $J_{3,4}$ = 5.53 Hz, $J_{2,3}$ = 6.99 Hz, H-3), 4.18 (1 H, dd, $J_{4,5}$ = 2.18 Hz, $J_{3,4}$ = 5.53 Hz, H-4), 4.40 (1 H, s, $J_{1,2}$ = 10.28 Hz, H-1), 7.13 (2 H, d, J = 8.03 Hz, Aromatic H), 7.43 (1H, d, J = 8.03 Hz, Aromatic H) ppm.

^{13}C NMR (125.77 MHz, $CDCl_3$): δ 26.54 (1C, C(**C**H$_3$)$_2$), 28.20 (1 C, C(**C**H$_3$)$_2$), 62.84 (1 C, C-6), 71.71 (1 C, C-2), 74.10 (1 C, C-4), 79.32 (1 C, C-3), 88.35 (1 C, C-1), 110.68 (1 C, **C**(CH$_3$)$_2$), 128.08 (1 C, Aromatic C), 130.05 (2 C, Aromatic C), 133.29 (2 C, Aromatic C), 138.71 (1 C, Aromatic C) ppm.

HRMS: m/z calcd. for $C_{16}H_{22}O_5SNa$ [M^+Na] 349.1086, found 349.1095

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9 APPENDIX

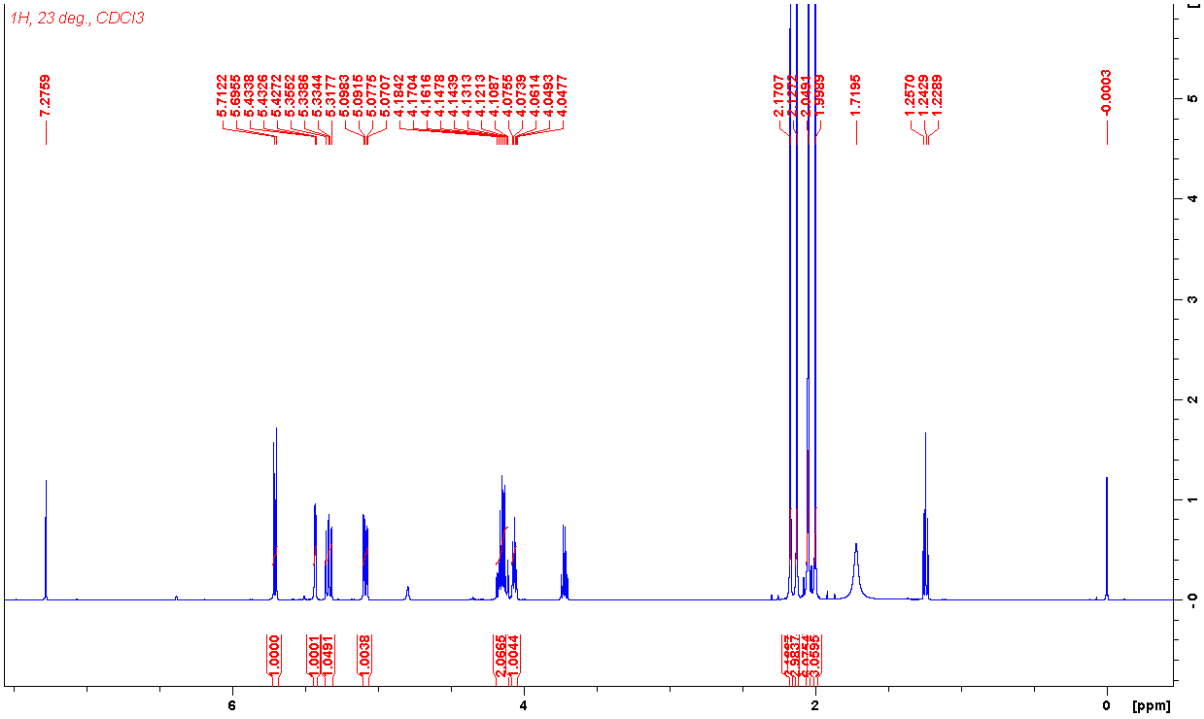

Figure 45 ^{1}H-NMR spectrum of 1,2,3,4,6-O-penta-acetate-β-galactopyranose measured at 23 deg. in $CDCl_3$.

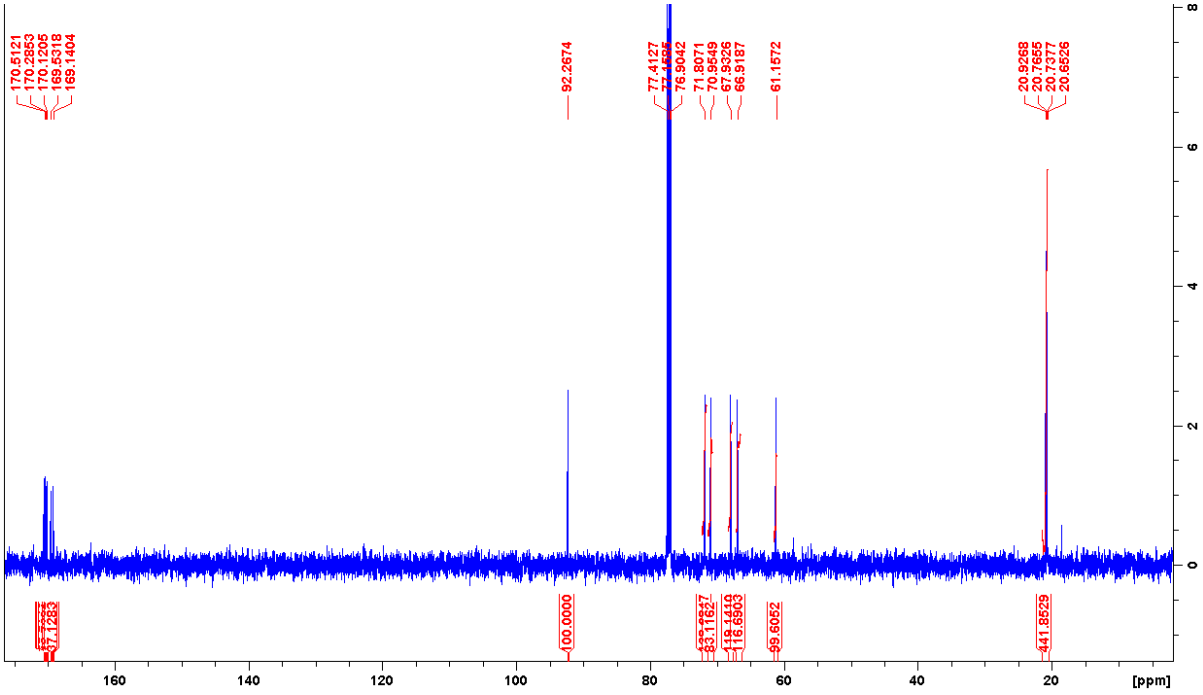

Figure 46 ^{13}C-NMR spectrum of 1,2,3,4,6-O-penta-acetate-β-galactopyranose measured at 23 deg. in $CDCl_3$.

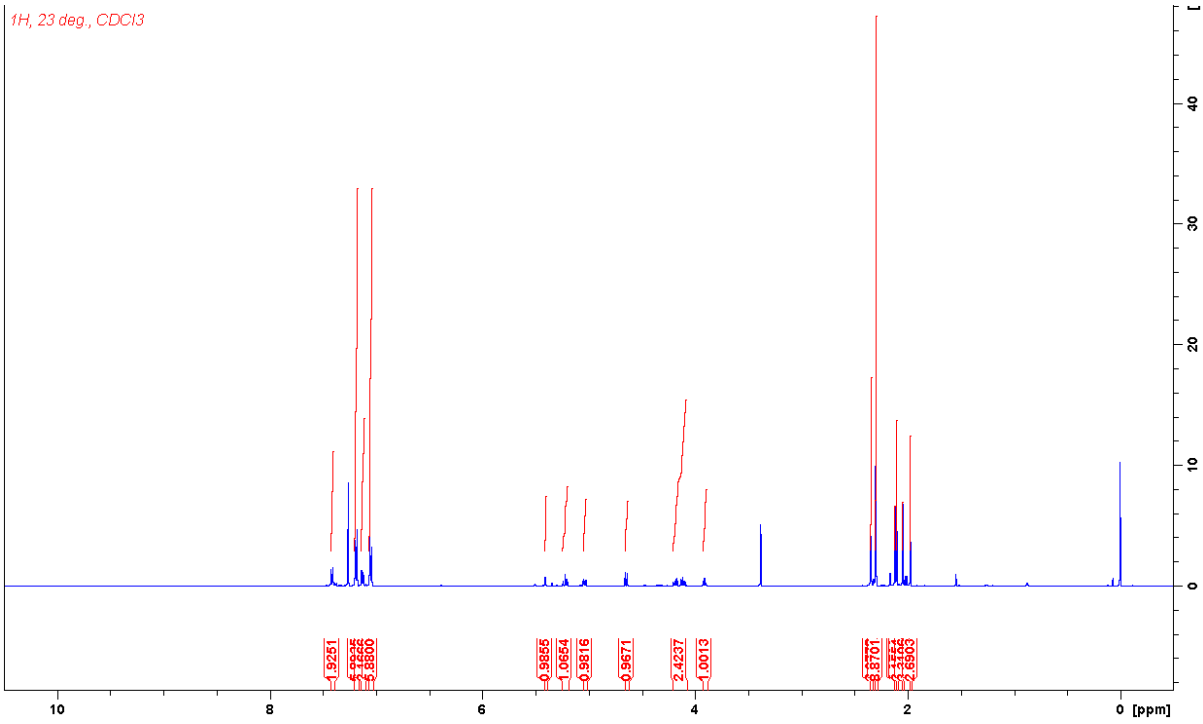

Figure 47 ^{1}H-NMR spectra of 4-Methylphenyl 1-thio-β-D-galactopyranoside measured at 23 deg. In $CDCl_3$.

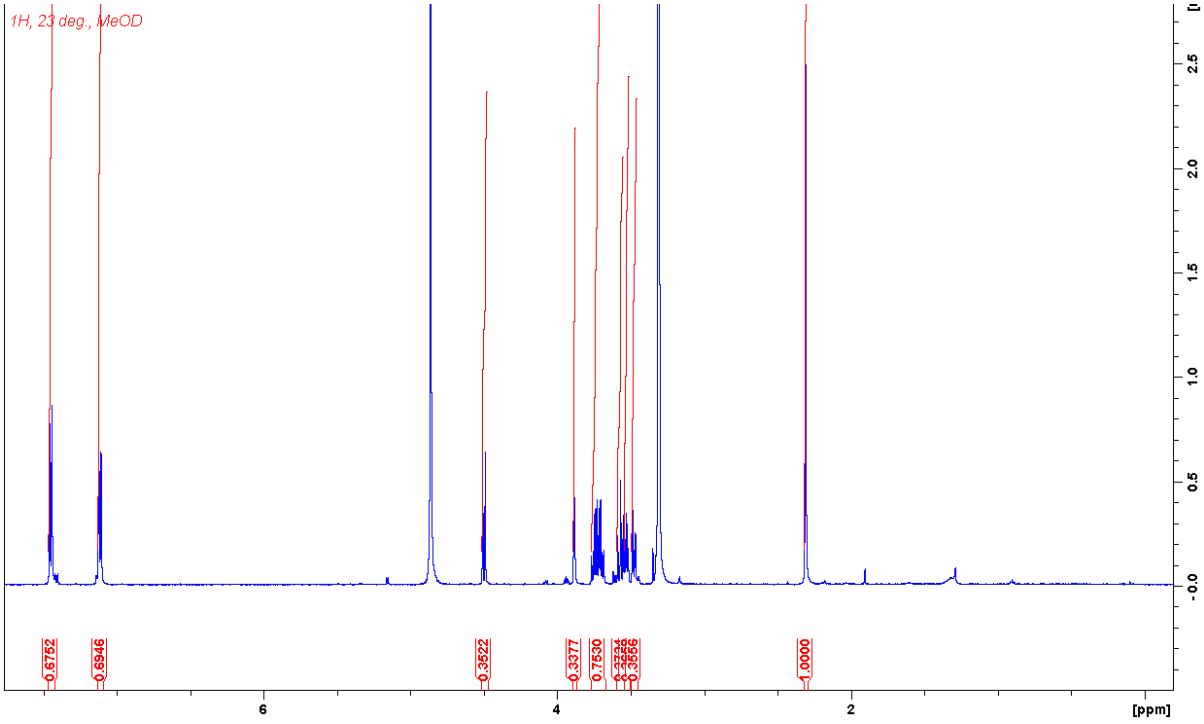

Figure 48 [1]H-NMR spectra of 4-Methylphenyl 3,4-O-isopropylidene-1-thio-β-D-galactopyranoside measured at 23 deg. In MeOD.

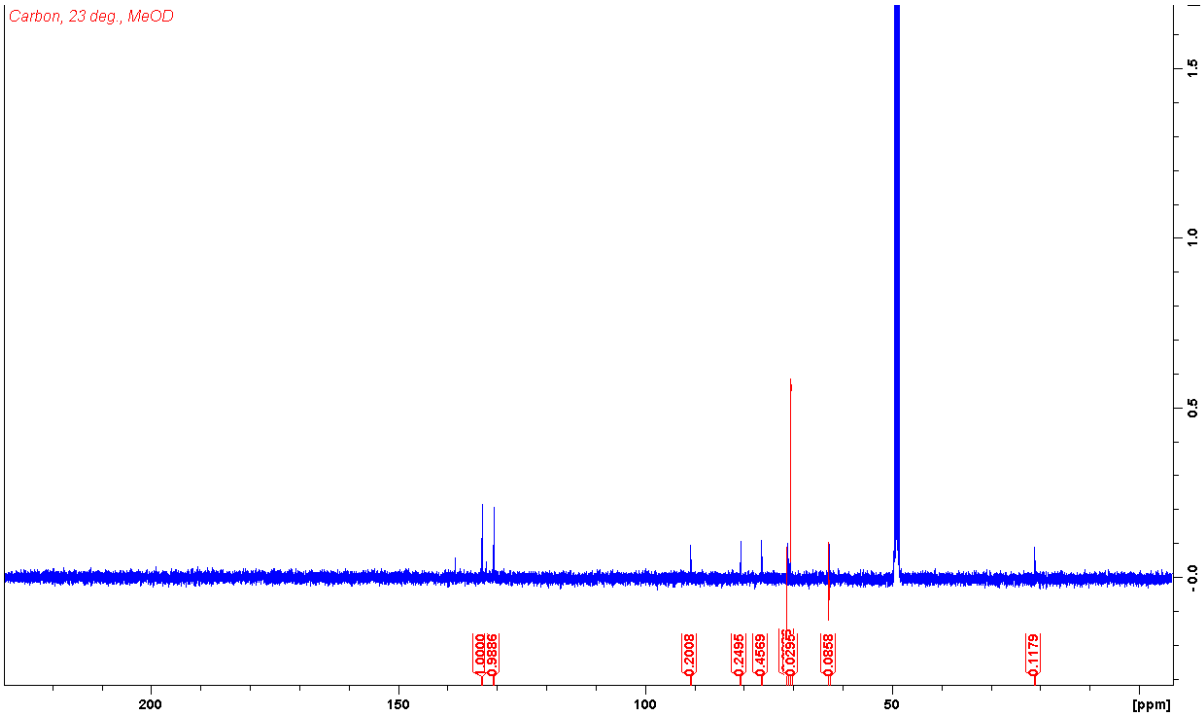

Figure 49 [13]C-NMR spectra of 4-Methylphenyl 3,4-O-isopropylidene-1-thio-β-D-galactopyranoside measured at 23 deg. In MeOD.

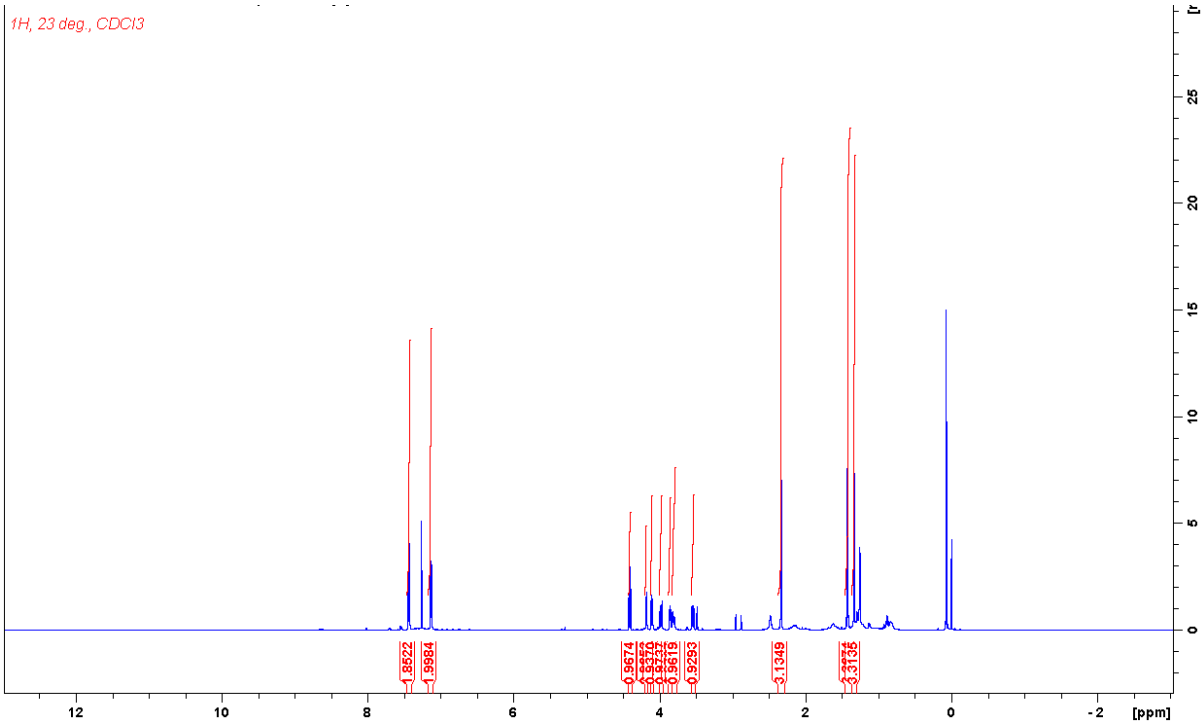

Figure 50 . ^{1}H-NMR spectra of 4-Methylphenyl 2,6-di-O-benzyl-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside measured at 23 deg. In $CDCl_3$.

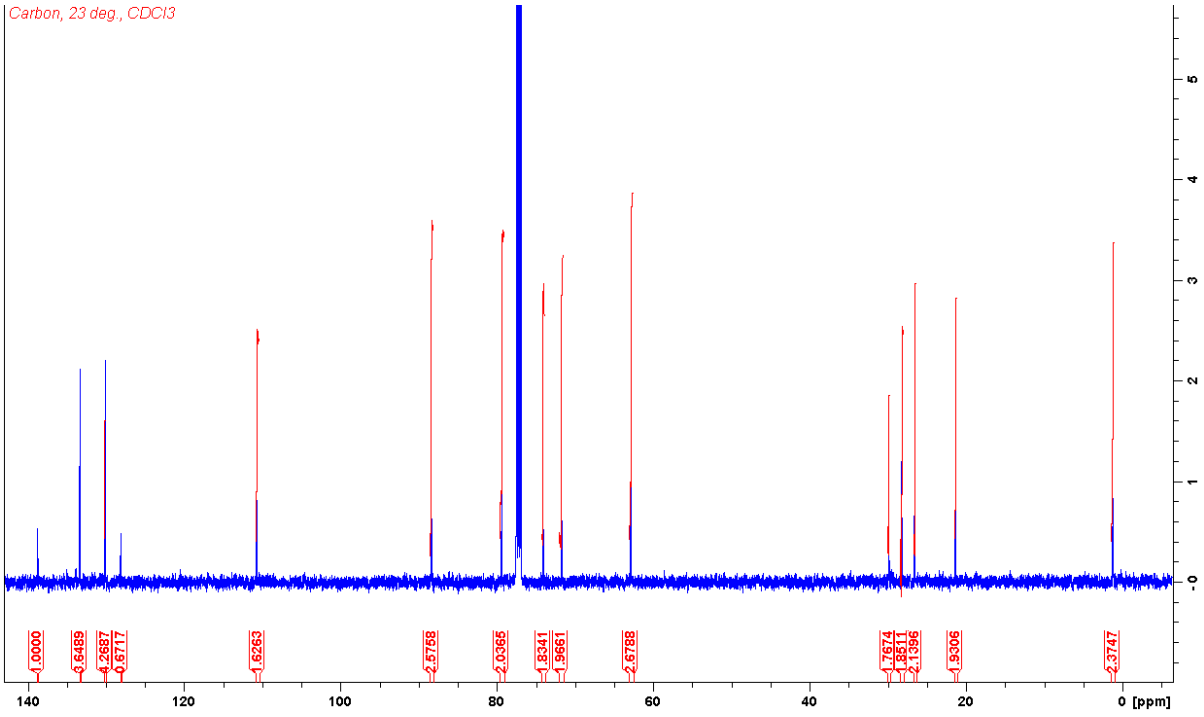

Figure 51 ^{13}C-NMR spectra of 4-Methylphenyl 2,6-di-O-benzyl-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside measured 23 deg. In $CDCl_3$.

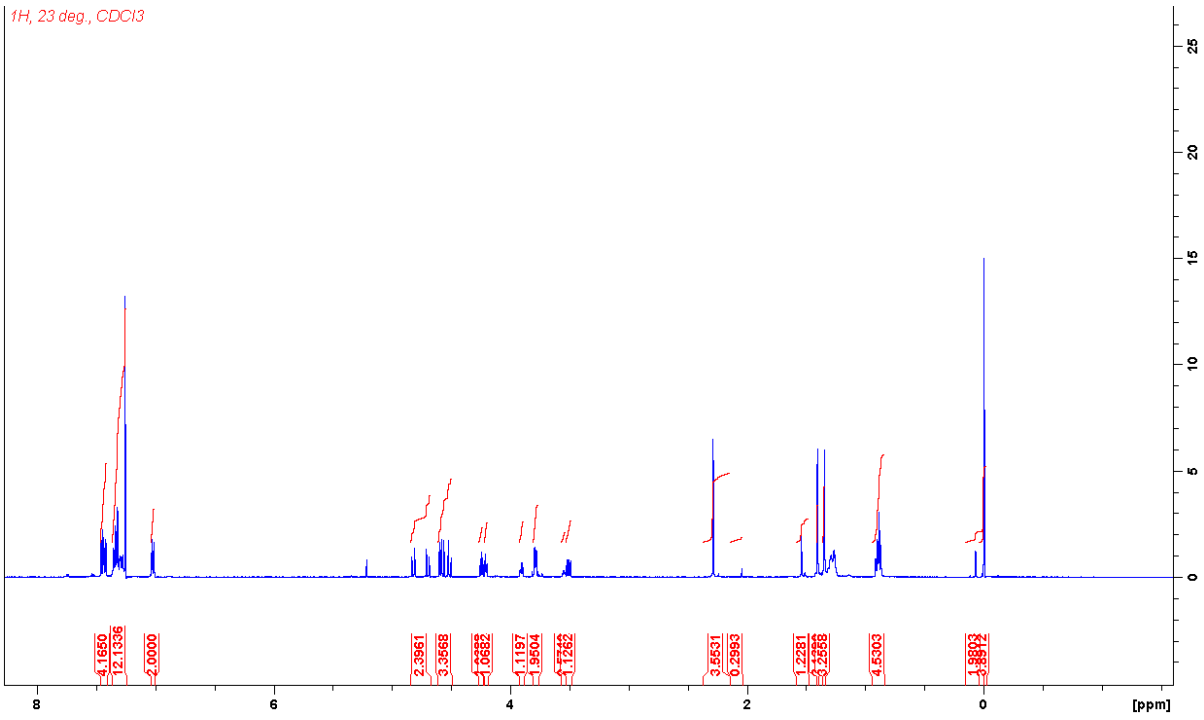

Figure 52 ^{1}H-NMR spectra of 2,6-di-O-benzyl-3,4-O-isopropylidene-D-galactopyranoside measured at 23 deg. In $CDCl_3$.

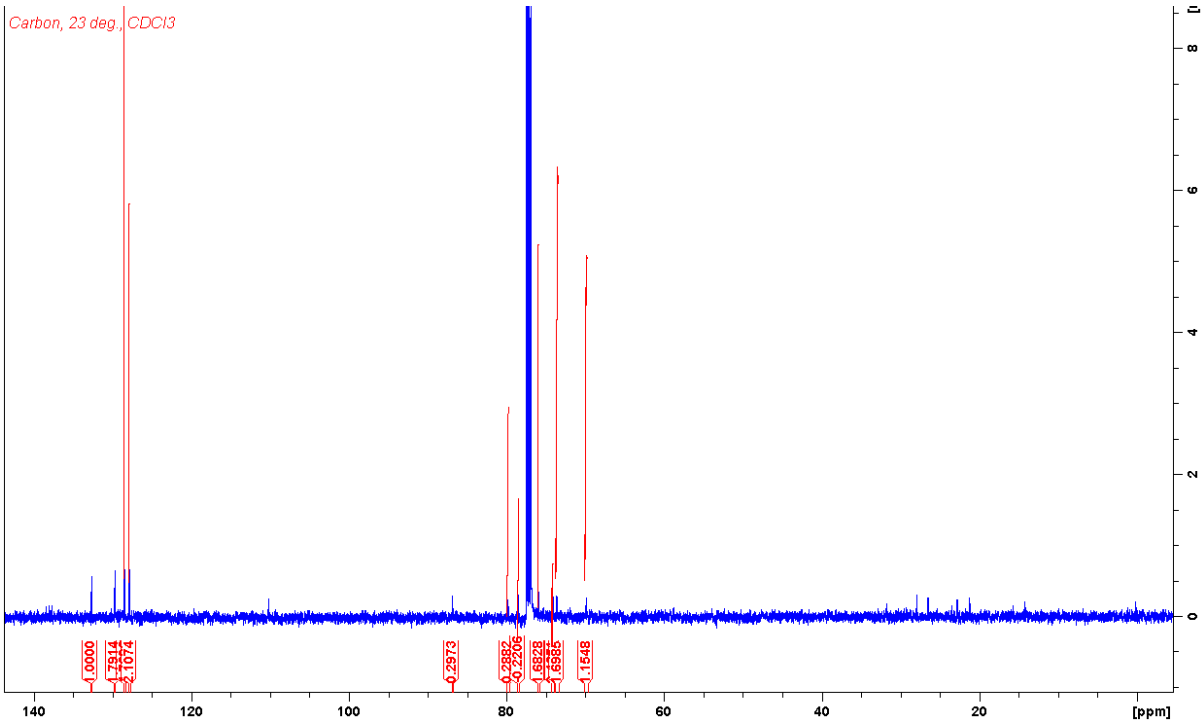

Figure 53 ^{13}C-NMR spectra of 2,6-di-O-benzyl-3,4-O-isopropylidene-D-galactopyranoside measured at 23 deg. In $CDCl_3$.

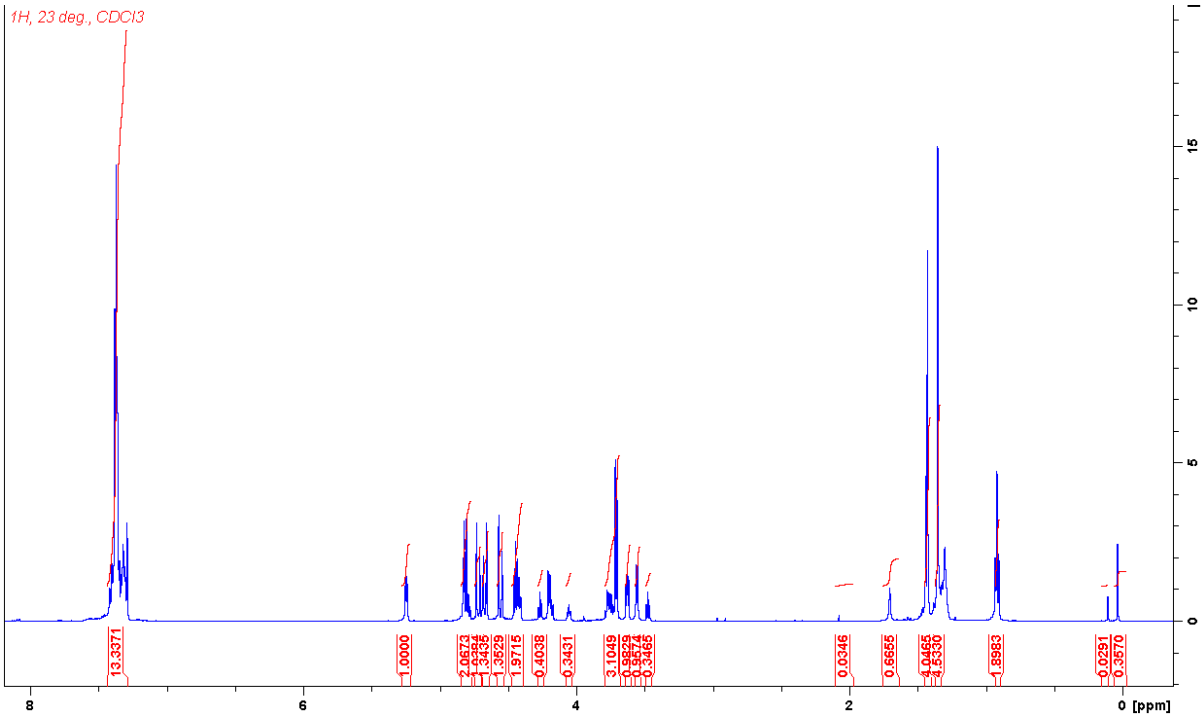

Figure 54 ^{1}H-NMR spectra of 2,6-di-O-benzyl-3,4-O-isopropylidene-D-galactitol measured at 23 deg. In $CDCl_3$.

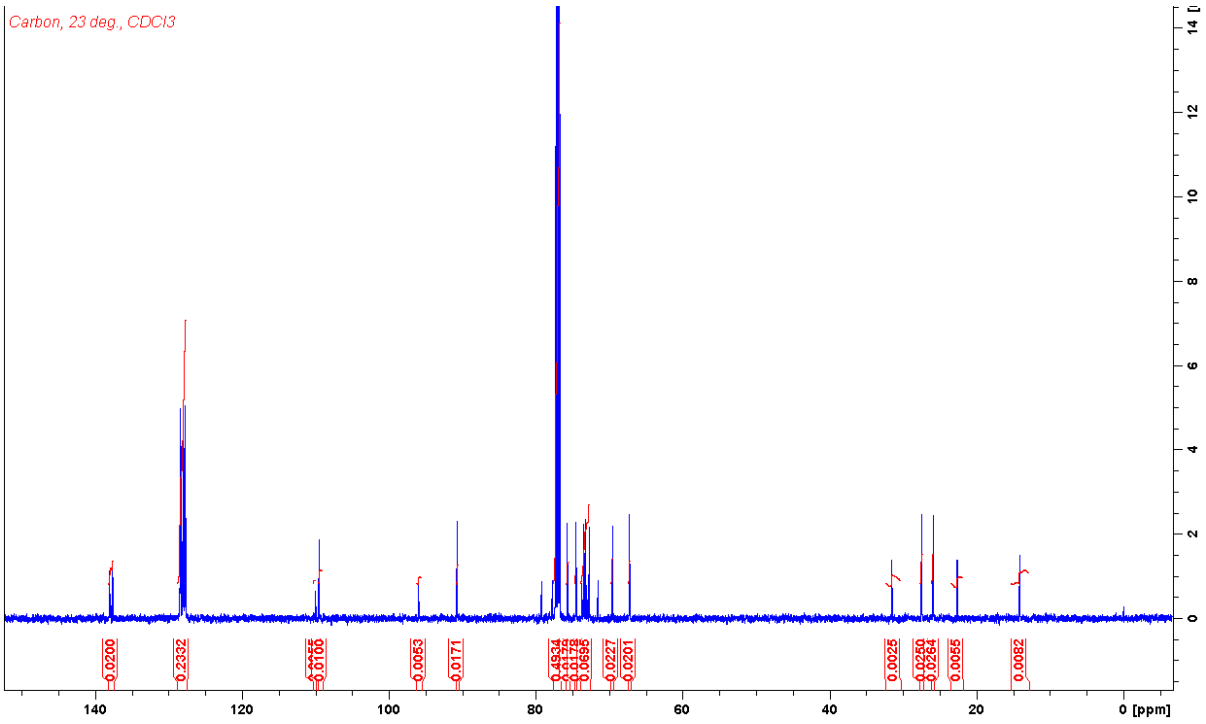

Figure 55 ^{13}C-NMR spectra of 2,6-di-O-benzyl-3,4-O-isopropylidene-D-galactitol measured at 23 deg. In $CDCl_3$.

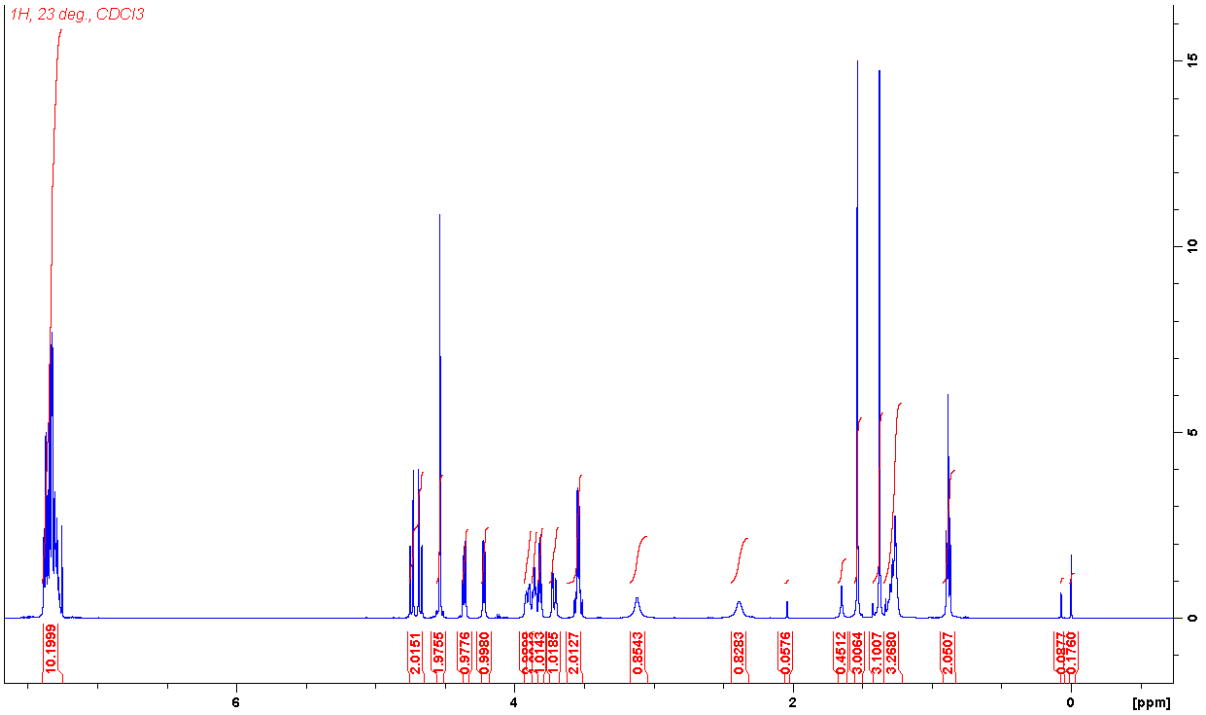

Figure 56 ^{1}H-NMR spectra of 2,6-di-O-benzyl-3,4-O-isopropylidene-1,5-di-tri-ethylsilyl-D-galactitol measured at 23 deg. In $CDCl_3$.

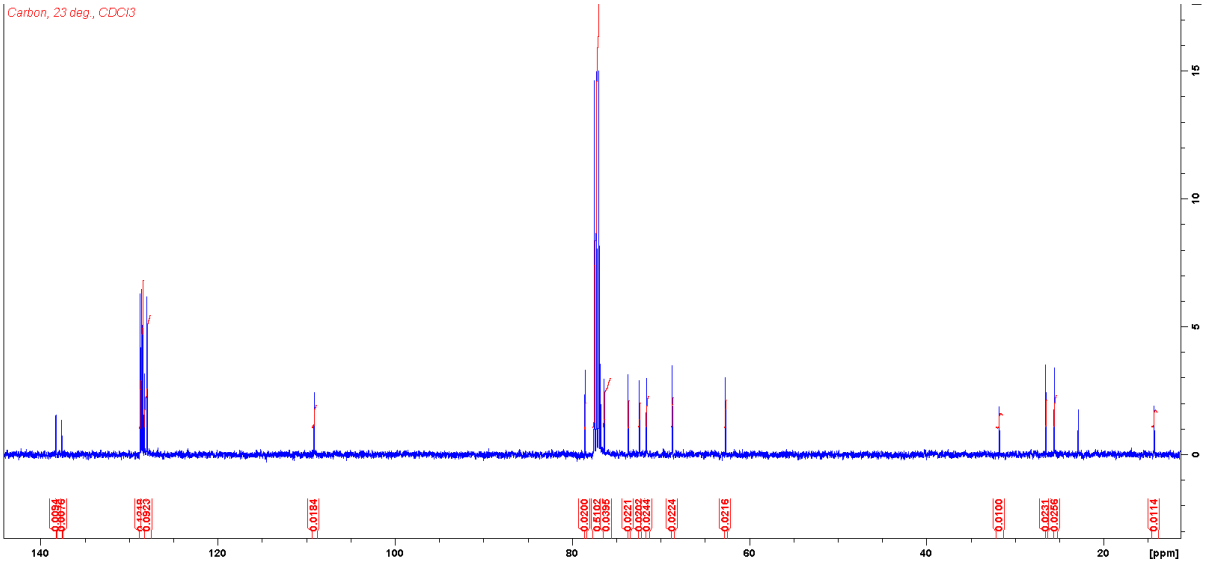

Figure 57 ^{13}C-NMR spectra of 2,6-di-O-benzyl-3,4-O-isopropylidene-1,5-di-triethylsilyl-D-galactitol measured at 23 deg. In $CDCl_3$.

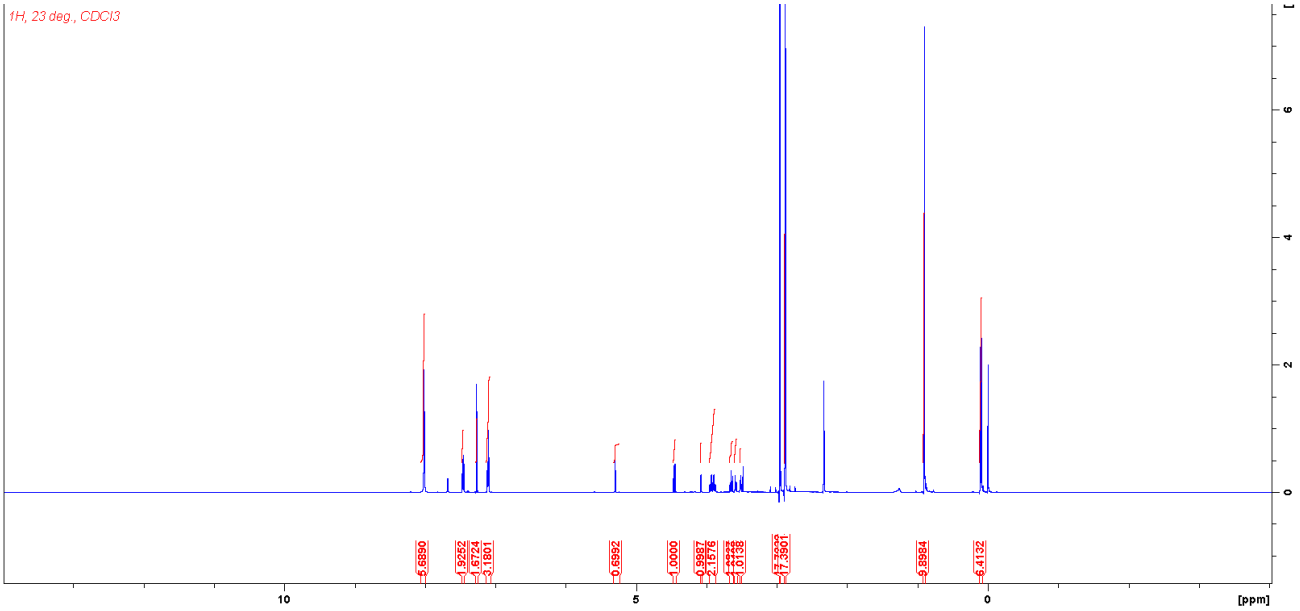

Figure 58 ^{1}H-NMR spectra of 4-Methylphenyl 6-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside measured at 23 deg. In $CDCl_3$.

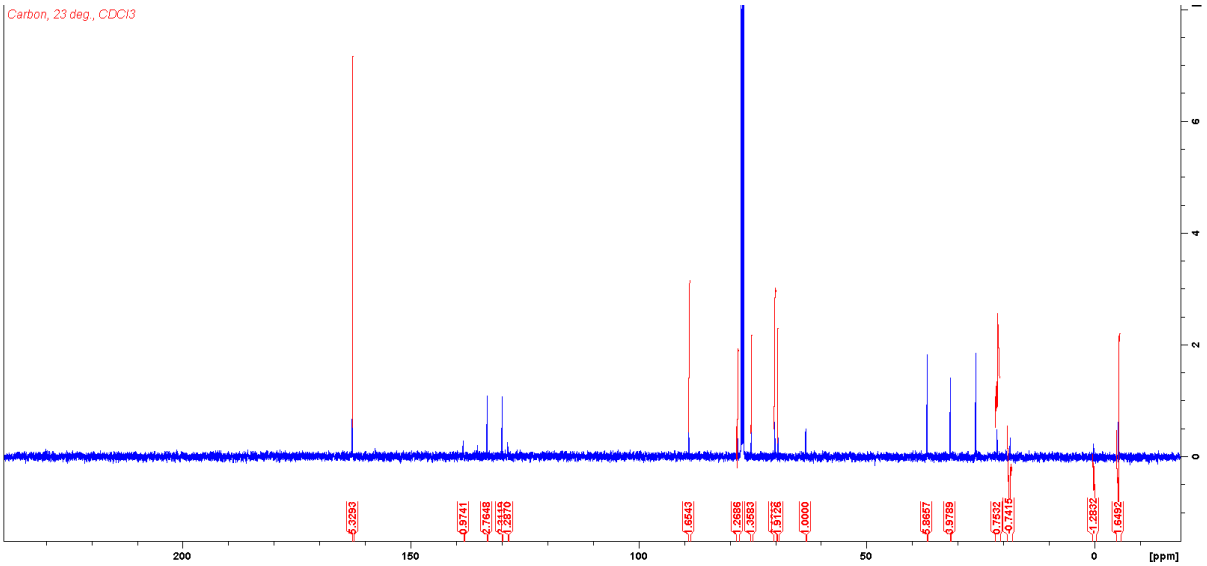

Figure 59 ^{13}C-NMR spectra of 4-Methylphenyl 6-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside measured at 23 deg. In $CDCl_3$.

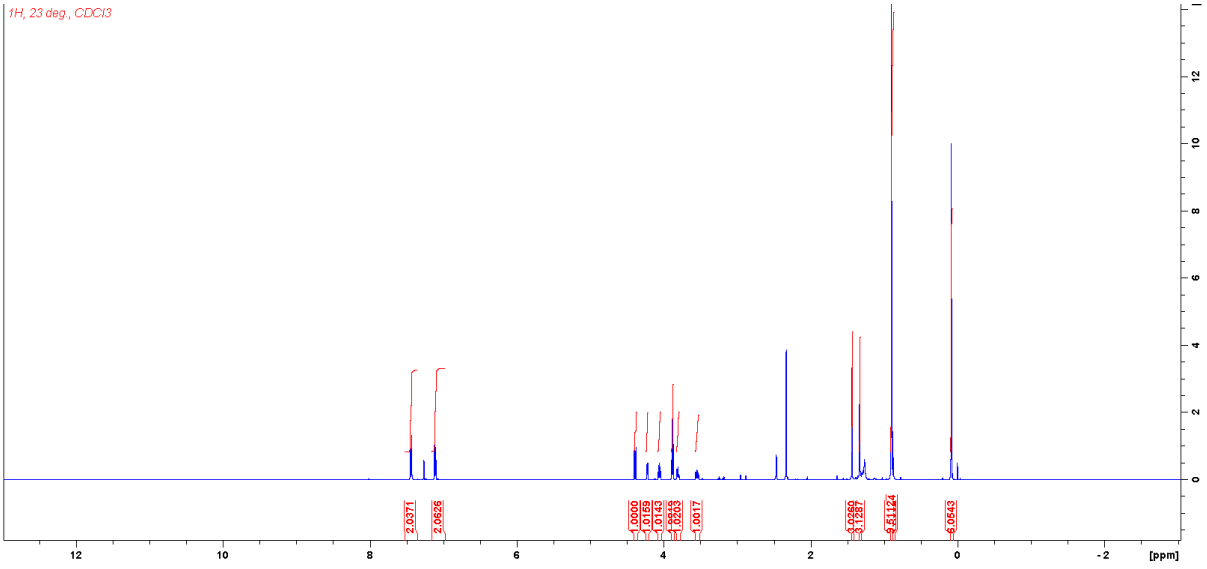

Figure 60 ^{1}H-NMR spectra of 4-Methylphenyl 3,4-O-isopropylidene-6-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside measured at 23 deg. In $CDCl_3$.

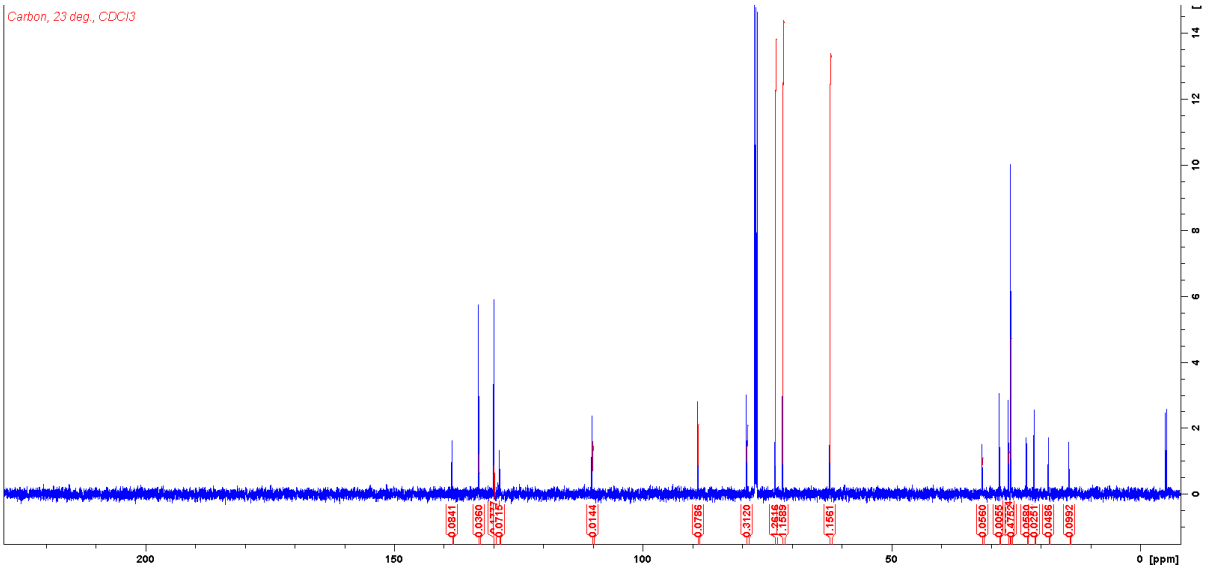

Figure 61 ^{13}C-NMR spectra of 4-Methylphenyl 3,4-O-isopropylidene-6-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside measured at 23 deg. In $CDCl_3$.